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CONGENITAL ABSENCE OF LIMBS IN TORTOISES OF THE GENERA TRIONYX AND EMYDA

BY

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The justification for writing this note has arisen from the fact that occasional absence of limb in the Gangetic tortoises was observed in more than one specimen. Two specimens of the genera Trionyx and Emyda showing such deformity were obtained during the course of the year The one, Trionyx gangeticus Cuv. measured 15″ × 12″ and lacked the right hind leg, while the other, Emyda granosa Schœpff measured 6″ × 4″ and was devoid of the right fore-limb. At the spot where the limb in question should have existed there was a dimple in the first and a fold of skin in the second tortoise. The two specimens were entirely normal in every other respect. In no case, however, was there any hint of a scar, thus showing that the absence of the leg may be congenital instead of being due to an accident.

A deviation from the normal growth of the paired limb occurs now and again throughout the Vertebrate series, as the fol owing brief account wil show

Fishes In fishes Hora 1921 (11) has described occasional absence of paired fins in a number of genera. A specimen of *Barilius barila* is described in which both the ventral fins were absent. In another species *B. dogarsinghi* the ventral fin of the left side was absent. In *Nemachilus kanjupkhulensis* the ventral fin of the right side was wanting and the other was abnormal. In *Rita rita* the right pectoral fin was lacking, the musculature was degenerated and the shoulder girdle was abnormal. In all these specimens of fish the limb-abnormalities were congenital defects as there was no trace of injury. Hora interprets them as mutations, or as due to some arrest in the growth of the organ.

Willey, 1920 (21), has referred to the absence of the ventral fins in a male Anna clava.

Jean Delphy, 1918 (4), describes cases of anomalous pelvic fins in *Cottus babulus*. In one there seemed only one fin perhaps due to coalescence of both the right and the left fins. In another there was a reduction of one fin almost to a vanishing point perhaps due to some arrest of development at an early stage.

Eigenmann and Cox, 1901 (9) and Brindlay, 1891 (2), have given description of abnormal fins in fishes.

Amphibians.—In the Urodele amphibian *Amblystoma punctatum* Harrison, 1921 (10), has shown experimentally that duplex and multiplex appendages frequently arise from the transplanted limb buds.

Banta and Gartner, 1914 (1), have published the results of some observations on accessory appendages and other abnormalities due to the action of centrifugal force on amphibian larva. There was usually only one accessory appendage to each animal though as many as four were noted. The appendages were usually lateral or dorsolateral in position. They were tail-like in external appearance

Abnormalities in hind limbs of *Rana* are described and experimentally probable variability of the organ reported by several notable workers. Reichenow, 1908 (16), reports on a number of abnormalities in hind limbs of young frogs *Rana esculenta*. One had only one hind leg, another three, and a third had four. Woodland (22) has furnished descriptions of some similar cases *R tigrina* in which a stalk bearing a pair of additional limb is attached to the thigh of the left leg. The aborted fused thighs of the additional pair of legs are represented by a small plate.

Harold Row, 1916 (17), describes a case of symmetry abnormal feet in *R. temporaria* which showed an absence of the first digit. There is no trace of mutilation.

Durkin, 1910 (6), has made experimental study of limbs in frog by extirpating the primordia of the limbs at a very early stage in the development of the animal. The amputation of one limb rudiment is usually associated with serious malformation in others. He explains the phenomenon as due to a very interesting effect on the development of the central nervous system brought on by the extirpation of the limb bud there being a developmental correlation between the nervous system and the peripheral organs. The affected nervous system exerts an influence on the other limbs causing a defective growth.

Lissitzky, 1910 (13), has induced duplicity by cutting the primordia of limbs in young tadpole.

Reptiles.—Duerden, 1922 (5), has observed in the South African lizard of the genus *Chamæsaura* that the three species show different degree of limb reduction. In *C. ænea* both pair of limbs are present but much reduced, in *C. anguina* both pairs are styliform and barely divided into two minutely clawed digits, in *C. macrolepis* the fore-limbs are absent and the two hind limbs are styliform and undivided He does not however think that the three species form a series showing stages in the direction of

further reduction of limbs but is of opinion that the age or the germinal factor concerned with the limb production is or has been in a highly mutative state.

Birds.—Zankewitsch, 1922 (29), has described abnormality in a Duck's wing A wing of *Anas boschas* showed on the ventral side in the region of carpo-metacarpus a hint of supernumerary limb. The feathering of the supernumerary part, which also bore two claws, inclined to be wing-like.

In a recent paper Roy, 1931 (18), has described heteromorphosis of the pelvic girdle, the presence of a pair of supernumerary hind legs and duplicity of cloacal openings in a domestic fowl. The pair of additional appendages were attached to the pygostyle by a peculiar conelike modification of the fused femur bones.

In another paper the writer in collaboration with Roy, 1931 (8), has given an account of the arrest in development of the right hind leg in a hen-feathered cock. The right femoral bone is represented as a small nodule attached to the acetabulum by the ligamentum teres.

Florence Peeble, 1910 (14), has made operation on the limb buds of chick. The results indicate "that when the tip of a young bud is grafted on the proximal portion of another limb it becomes a part of the appendage to which it is attached instead of retaining the character of the part it is destined to become. No regeneration of the limbs takes place after the removal of the buds."

Mammals.—An interesting account of congenital absence of both hind legs in an adult pig is given by Sumulong, 1926 (20). Carreon, 1919 (3), has reported absence of hind legs below femur in a full term pig. He explains the abnormality as due to some physico-chemical interference very early in the development of the pig.

Khirkham and Haggard 1916 (12) described the structure of a three-legged Kitten—the left fore-limb being

apparently absent The limb bud he thinks had encountered some obstacle and checked its growth.

It will be seen from the above that reports on a number of abnormalities of the appendages in Vertebrates have been contributed by good many observers, but no account seems to be extant in so far as the limb-abnormalities of the Chelonia are concerned. The writer therefore takes the opportunity of describing the anomalipeds.

The photograph (Fig. 1) shows the ventral aspect of the larger tortoise (Trionyx gangeticus Cuv.) without the right hind leg. The plates of the plastron are perfectly normal and so are those of the carapace. The plastral callosities are large and normal. The visceral organs lie in proper situation without any trace of abnormality except the right leg, which is apparently absent. The muscles are extremely degenerate in the particular region where the right leg should have been. The right acetabulum is very imperfectly got up and convex instead of concave, a structure suggestive of developmental arrest of the leg and subsequent fusion with the girdle. The rest of the pelvic girdle is free from deformity. The blood vessels and the nerves are on a reduced scale only supplying the degenerate muscles and connective tissue of the right leg.

The younger specimen Emyda granosa Schœpff (Figs. 2 and 3) shows outwardly no indication of the existence of the right fore leg. The skin covering the spot is folded into wrinkles and on dissecting out the animal it was found that the muscles and bones of this limb showed great retrogression in development. The bony part being reduced to a small nodule lay deeply imbedded in the flesh. This piece of bone measured $0{\cdot}5'' \times 0{\cdot}5''$ and was attached to the right glenoid of the pectoral girdle by a ligament. The girdle is well-formed and normal.

The probable cause of the limb abnormality is due to some injury to the anlage or the germina factor concerned

with the production of the un at a very ear v sta e of development. Stockard, 1921 (17), Patterson, 1907 (13) and several other observers have found the developmental interruption of the organ to exist in many animals in connection with other abnormalities besides the limbs. At a very early stage of the development the central nervous system plays a very important rôle in co-ordinating the development of limbs and other organs, and any disturbance to the central nervous system must necessarily cause serious defects in the body-building. Reciprocally, as Durkin has shown, that hindering of the development of an organ is followed by abnormal development in the whole central nervous system and from the affected nervous system an influence is exerted on other parts of the body causing deformities. The disturbance may be brought about by inadequate supply of oxygen, food, or it may be caused by external shock or injury. According to Banta and Gartner, "the hereditary determiners for development work out their destined end only when maintained in certain appropriate relation."

In the end the writer wishes to express his sincere thanks to Professor D. R. Bhattacharya for help and criticisms. The work was carried out under his direction in the Department of Zoology of the University of Allahabad.

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THE TRANSFERENCE OF GOLGI BODIES FROM THE FOLLICULAR EPITHELIUM TO THE EGG IN CERTAIN INDIAN SNAKES

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INTRODUCTION

My work on the oogenesis of certain Indian snakes revealed many points of interest, not the least interesting of which has been the phenomenon of the transference of Golgi bodies from the theca and the follicular epithelium cells to the egg. The well-developed Ophidian oocyte has a fairly thick layer of peripheral Golgi bodies: such a condition, however, is not novel and is easily demonstrated in the earlier and later stages of most vertebrate and at least some invertebrate eggs. The presence of a second type of Golgi bodies in the follicle cells and in the outermost confines of a fowl's egg led Brambell in 1924 to express the view that a good portion of them is derived from the follicular epithelial cells as a result of an inward migration—simultaneously with Bhattacharya who observed a similar phenomenon in tortoises. Since then the latter author and his pupils, of whom the present writer is one, have examined a large number of vertebrate eggs and reported the infiltration of Golgi bodies, as they have called it, in tortoises lizards birds fishes and frogs The aim o the

present paper is to add to the types in which the infiltration of Golgi bodies from the theca and the follicular epithelium cells to the egg has been observed, and to indicate, so far as possible, the manner and the significance of the process.

It is my pleasant duty to record my obligation to Dr. D. R. Bhattacharya, Professor of Zoology in this University, to whose original idea of Golgi infiltration the present paper owes its origin, for his unfailing courtesy in help and guidance.

MATERIAL AND TECHNIQUE

Ovaries were taken and fixed from the following five types of snakes—Zamenis mucosus, Eryx conicus, Tropidonotus stolatus, Tropidonotus piscator, Gongylophis conicus. All possible precautions as recommended by Gatenby and Cowdry (8) during the period of transference of the ovary from the body of the animal to the fixatives were taken. Of the large number of fixatives which were employed the following gave good results in showing clearly the egg membranes and demonstrating the Golgi bodies in various structures :

(1) DaFano's Cobalt-Nitrate Method.

(2) Cajal's Uranium-Nitrate Method.

(3) Ludford's latest modification of the Osmic Acid Method.

(4) Flemming without Acetic Acid.

In the case of (1) and (2), the sections were toned with 1 per cent Gold chloride, 5 per cent Hypo and 3 per cent Ammonium sulphocyanide. The osmiophilic intensity of a snake oocyte being much less than is the case in tortoises and other vertebrate eggs, it was not found necessary to bleach the sections by Henneguy's method

EGG MEMBRANES

The earliest oocytes of the snakes examined are lodged in investments of thecal tissue, which unlike their anlage in pigeon develop as a single layer and are not divided into Theca interna and Theca externa. As the oocyte grows, the follicular epithelium is formed as a multi-layered structure and this condition is assumed at an early stage of development. The individual cells are at first small but soon grow into what is usually a middle layer of large oval cells having a nucleus and a prominent nucleolus, and a few layers of smaller cells on the inner and outer sides. The cells next to the zona radiata are specially small and often crowded together.

In between the follicle cells and the egg, the only structure visible is a narrow faintly striated layer which usually does not take much stain and is not quite transparent. This, without doubt, corresponds to the zona radiata so well developed in tortoises and visible as a single layer in lizards and birds. There is, however, no trace of any fibrillæ layer found in tortoises, nor could any well-formed 'limiting membrane' as noticed in other reptiles and birds, be distinctly made out. The zona radiata usually develops at a late stage of oocyte growth.

GOLGI MIGRATION

In well-developed oocytes of Eryx conicus and Tropidonotus stolatus, Golgi bodies are present in the theca and in the follicular epithelium in large numbers (Figs. 1 and 2). In Ludford and F.W.A. preparations they appear as dark granules or as crescents. Often many of the granules are aggregated together, exactly in the same manner as they do in the extreme peripheral region of the egg. The actual transference of the Golgi bodies from these layers takes place in

isolated units each individual granule working its way only inwards. No chains of migrating Golgi bodies or regular passages, such as has been reported in tortoises by Bhattacharya (1), or little lumps as was first indicated in fowl by Brambell (6), or as described in lizards and in birds by Datta and Das (3) respectively, could be observed in any of the five Ophidian types examined.

In a well-grown oocyte of Zamenis mucosus, Golgi bodies are present in fairly large numbers in the theca as well as in the follicular epithelium cells. In the latter the dictyosomes often aggregate closely around the nucleus on the side nearest to the zona radiata. Thence they seem to be continually migrating, in a measured degree as it were, to the outer layer and finally to the cortical region of the egg. These migrating Golgi bodies are commonly seen almost at all places in a well preserved zona radiata. A very clear instance of their crossing the last limits of the zona radiata and almost stepping into the confines of the egg is shown in Fig. 5, which is a Cajal preparation of Gongylophis oocyte. Probably by mere coincidence or as a result of rotation the Golgi bodies have come on the innermost area of the zona radiata simultaneously throughout the circumference of the egg and are caught in the act of entering it.

Figure 4 is an earlier stage of the oocyte of Tropidonotus piscator. Only one definite layer of follicular epithelium cells is prominently seen. A few smaller cells perhaps are also present and the boundaries of the individual cells are not very well defined. The zona radiata has not yet developed. The number of Golgi bodies present in the follicle cells is immense and their passage to the periphery of the egg wholesale, so much so that there is hardly any dividing line visible between the oocyte and the follicular epithelium. The cortical region of the egg as well as the follicular cells are thick with Golgi

bodies and appear very dark in a DaFano preparation. No definite method of transference can be made out here, for there seems to be none, but the fact of the transference itself is too apparent. A similar stage of Zamenis mucosus shows a many-layered follicular epithelium of small cells, the zona radiata unformed, but the activity of infiltration equally intense.

DISCUSSION

The condition of the egg membranes is almost similar to that described by Mlle. Loyez in 'Ophidians' where (1) the follicular epithelium is said to contain 'small cells which can divide mitotically, (2) intermediate cells arise from the differentiation of the small cells in the inner layer of the follicle, (3) large pear-shaped cells result from the development of the intermediate elements' The large pear-shaped cells are, however, well-marked features of the follicular epithelium of well advanced oocytes : other smaller cells are present in all stages of development and their division into smaller and intermediate cells in the form of definite layers can hardly be justified.

The behaviour of the Golgi bodies with respect to their migration is almost similar in all the five types examined. In all of them infiltration becomes active and marked in certain stages in the development of the oocyte, and the onset of the phenomenon is noticed specially in the later stages. An early oocyte even with a well-formed follicular epithelium shows hardly any Golgi bodies in the latter structure, much less any signs of infiltration. This seems to be directly in contrast with the condition in fowls where according to Brambell (6) "the process of intrusion of elements from the follicle ceases at the time when the one-layered follicle becomes many-layered and commences to secrete zona striata

The Golgi bodies that pass out from the follicle cells are fairly large irregular bodies and not unoften two or three elements are inextricably fused together. This is specially the case in Eryx conicus and Tropidonotus stolatus where the tendency to aggregate in clusters is most marked. It is, therefore, obvious that a zona radiata with definite channels and a fibrillar layer with similar canaliculi should be more a hindrance than help for the passage inwards of these bodies, transmission through which is only possible for dust-like particles.

Unlike the case in fowls the appearance of the zona radiata does not seem either to commence or to stop the infiltration activity of the follicle cells. Only in the absence of this layer the transmission of the Golgi body is more prolific and haphazard. Miss Thing (9) has stated that "the structure of the zona pellucida," and this term includes zona radiata, "presents a condition most favourable for the conveyance of nutritive material from the epithelial area in contact with the maternal capillaries to the actively growing and extending yolk." Considering the part played by the zona radiata in the oocytes of snakes, in this connection, it seems possible to suggest that this layer acts not only as a vehicle of Golgi transmission in virtue of the incidence of its position, but also as an active medium regulating the inflow of the Golgi bodies which would otherwise filter down in utter disregard of the metabolic needs of the growing egg. For after all as Waldeyer, Mlle. Loyez and others have pointed out the transmission of the Golgi bodies from outside to the egg must have a definite role to play in the economy of the latter body and it is natural to suppose that this process should have greater chances of success when the inflow is measured and regulated than otherwise.

The infiltrating Golgi bodies have nothing to distinguish them either from those elements that are in the follicle cells or from those found in the oocyte

They settle down as in other animals in the cortical region which becomes dark and thick being packed full of them, particularly in DaFano and Cajal preparations. This is specially the case prior to the formation of the zona radiata. After the emergence of this layer the Golgi bodies continue to swell, aggregate or even fragment in the cortical layer and are gradually used up.

SUMMARY

1. In a fully grown oocyte the egg membranes, besides the theca, consist of (*a*) a many-layered follicular epithelium and (*b*) a comparatively narrow strip of zona radiata. The latter layer develops only in the later stages

2. The infiltration occurs only at certain late stages in the development of the oocyte. The Golgi bodies make their way inwards, when the zona radiata is present, in the form of isolated, though often fairly large and irregular elements In the absence of the zona radiata the infiltration is wholesale and the amount of the Golgi bodies passed is excessive.

3 No distinction was observed between the infiltrated Golgi bodies of the follicular epithelium and those present originally in the oocyte.

4 The tendency of the Golgi bodies to aggregate closely in groups or in patches of dictyosomes in the periphery of the oocyte was equally noticeable in the follicular epithelium cell.

5 The remarkable unanimity of all the five types of snakes examined with respect to their egg membranes as well as in the behaviour of their Golgi infiltration has been noticed.

6. One of the possible functions of the zona radiata has been suggested.

LETTERING

V	.	Vacuole.
M.		Mitochondria.
Th.	..	Theca.
F E	...	Follicular epithelium.
Z R	...	Zona radiata.
G B.	.	Golgi bodies of the oocyte.
g. b.	.	Golgi bodies of the theca, follicular epithelium, and the zona radiata.
G G		Closely aggregated groups of Golgi bodies
Inf G		Infiltrating Golgi bodies

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EXPLANATION OF THE FIGURES

Fig 1 — Golgi bodies present in the follicular epithelium as well as in the oocyte Many of the individual elements have aggregated together forming little patches. A distinct zona radiata is present through which the infiltrating Golgi bodies are seen Eryx conicus Ludford's latest osmic fixation. Altmann stain.

Fig. 2. —Golgi bodies present in the theca, follicular cells and the oocyte A fine zona radiata is visible with yolk granules

of C[illegible]ents [illegible]r [illegible]ced [illegible]op[illegible]n
[illegible]s[illegible]s[illegible]n[illegible]w [illegible]n[illegible]k[illegible]s[illegible]l[illegible]l
F.W.A. fixation. Crumpy-Kull stain.

Fig. 3.—Large pear-shaped follicular cells with smaller ones on the sides. A distinct zona radiata. Infiltrating activity slow and measured. Full-grown egg of Zamenis mucosus. Ludford's latest osmic fixation.

Fig. 4.—Follicular epithelium hardly more than one-layered. No trace of zona radiata. Infiltration haphazard and excessive. Tropidonotus piscator oocyte. Dal'ano fixation. Saffranin and Light-Green stain.

Fig. 5.—Infiltrating activity at its highest though the presence of zona radiata seems to regulate the process. Large pear-shaped cells in the central follicular layer. An advanced egg of Gongylophis conicus. Cajal fixation. Toned with Gold chloride and Hypo.

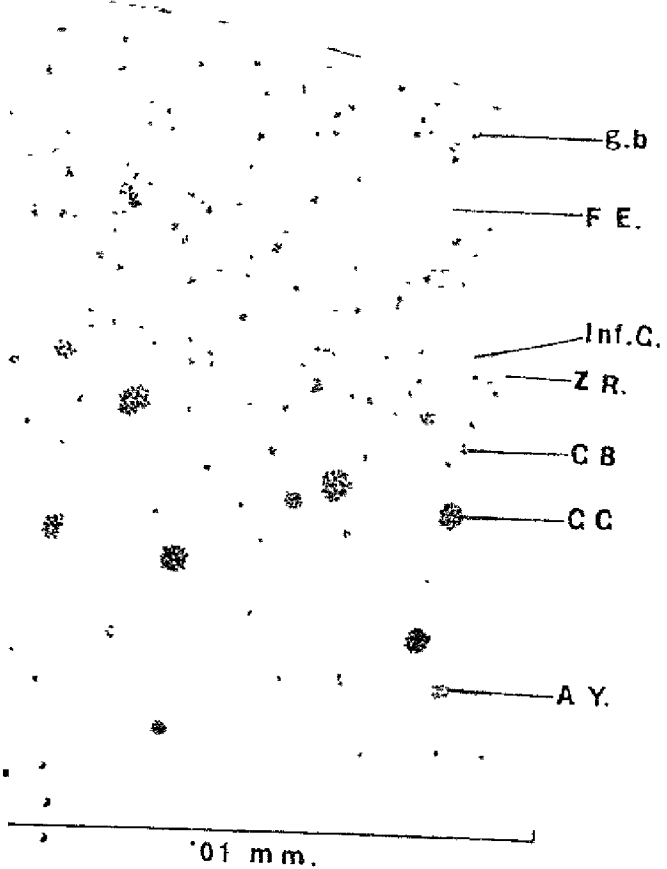

Fig 1

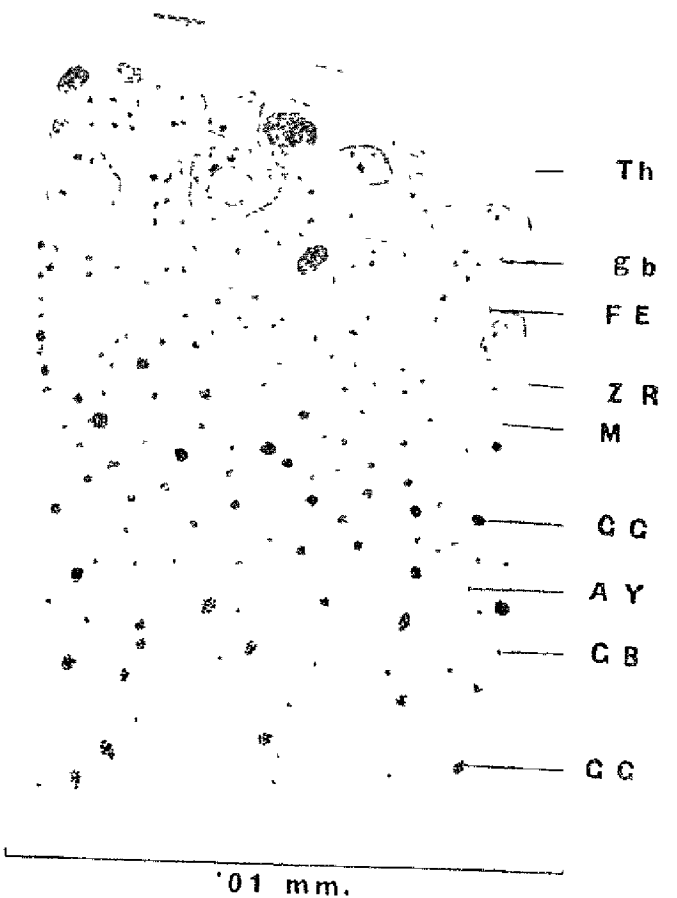

Fig 2

Plate 1

Plate 4

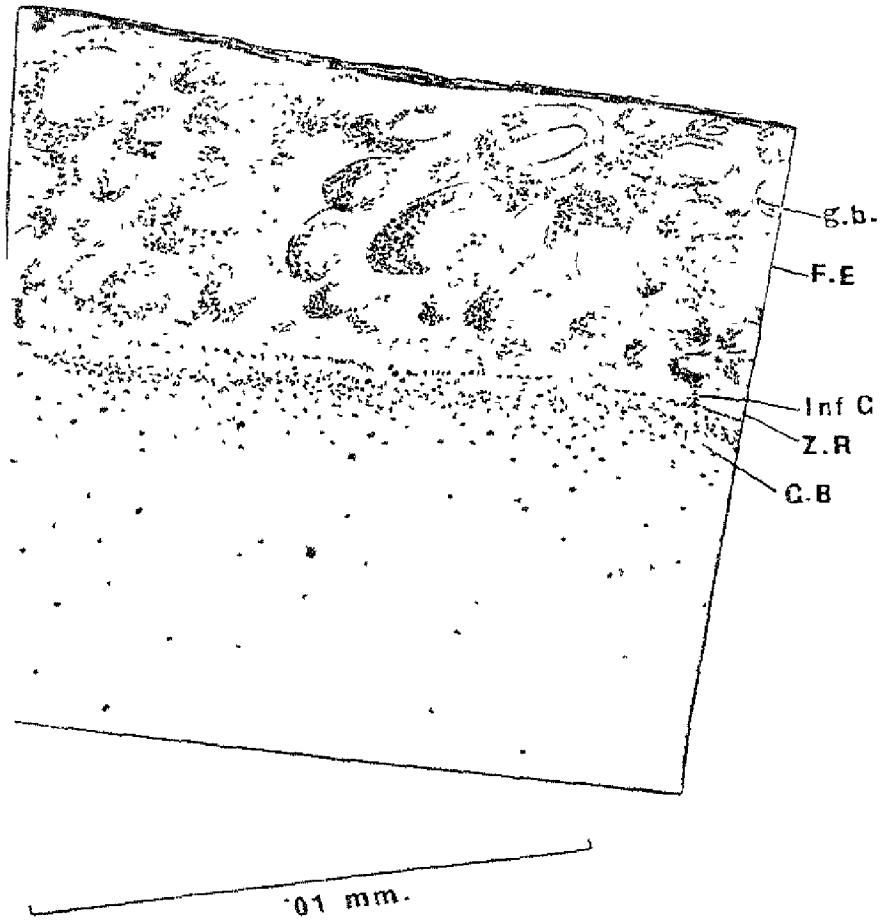

Fig. 5

NOTES ON THE STRUCTURE OF THE GONAD AND DEGENERATION OF ONE HIND-LIMB IN A HEN-FEATHERED COCK

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INTRODUCTION

In a previous paper (16) one of us (G. N. R.) has described heteromorphosis of the pelvic girdle, the occurrence of a pair of supernumerary pelvic appendages and the duplicity of cloacal openings in a domestic fowl. In the present communication we propose to record the results of the histological study of the testes of a hen-feathered cock and to describe its defective hind-limb—a deformity noted, we believe, for the first time

EXTERNAL CHARACTERS

The description is based on the observations of a fow possessing only one leg—the left leg, and having feathers

I sc on l t e
was after dissection that we recognised its m

Fig 1

Text Fig 1 Photograph of the hen-feathered co
t It has only one leg- the left and has
dary sex c arac rs r s

The only measurements are as follows

Length, measured from the tip of the beak to the extremity of the tail .	15 inches
Breadth, round the wings	9 inches
Length of the wing	9 5 inches
Length of the normal leg ..	13 inches

(measured from the pelvis along the hinder border up to the tip of the third digit).

Length of the femur of the left leg ...	3 inches
,, ,, Tibio-tarsus . .	4 5 inches
,, ,, Tarsometatarsus ..	3 inches
,, ,, 1st digit	·75 inch
,, ,, 2nd digit . ..	1·4 inches
,, ,, 3rd digit	2 inches
,, ,, 4th digit . .	1 5 inches

For the preparation of the slides of testes, DaFano's cobalt nitrate formol was used for fixation and subsequently the tissue was impregnated with 2 per cent silver nitrate. Sections were cut by paraffin method, toned with gold chloride and stained in iron haematoxylin and eosin.

THE STRUCTURE OF THE TESTES

The investigations of Benoit, 1921 (1), Firket, 1914 (4), Loisel, 1902 (8), Morgan, 1920 (11), Nonidez, 1920 (12), Pezard, 1918—22 (15), Shattock and Seligmann, 1904 (17), and others have definitely established the fact that the condition of plumage and the development of the secondary sexual characters of a fowl are governed by the internal secretion of its gonads But there still exists diversity of opinion as to the particular cells which produce the secretion in the organ. Boring and Morgan (2) have shown that the condition of plumage in cocks of Sebright bantam breed in which all males are hen-feathered, is dependent upon the presence of certain interstitial cells

of [illegible] regarded to inhibit cock-feathering. While on the other hand Fell (3) has tentatively put forward a hypothesis that the feathering in the sexes of the fowl might well depend upon the amount of lipoids contained in the blood.

"If the lipoid content is greater than a certain amount, say X, the plumage will be of the female and if less than X of the male type. In the normal female the amount would be above X, ovariotomy would cause it to fall below X, and the bird would become cock-feathered. In the normal male it would be below X and castration would cause a slight fall, as perhaps expressed by the more luxuriant plumage of the capon. In the case of the hen-feathered Sebright and Campine Cocks the fat concentration would be slightly above X, castration would cause it to fall below and the male plumage would be exhibited. (Fell, H. B., Brit. Journ. Exp. Biol., Vol. I, No. 3, 1924, p. 307.)

In his extensive series of memoirs on the studies of gonads of the fowl, Jose, F. Nonidez (12) has pointed out that in most hen-feathered males the interstitial cells become fat-laden and agrees with the hypothesis advanced by Fell in so far as it assumes that the gonads of either sex stimulate the production of lipoid material in the blood. He remarks—"Although the problem has not been sufficiently studied, the few observations 7 and 13 published thus far are consistent with the hypothesis."

We are unable to determine the lipoid contents in the blood of the bird in question, but on careful histological study of the testes we have been able to ascertain the occurrence of abundant aggregations of large-sized interstitial cells of the ovarian type which according to Morgan and Boring control the expression of secondary sexual characters and also the formation of plumage.

The Seminiferous Tubules.—The testes appeared quite normal [illegible]. The normal epithelium is made up

cells towards the periphery of the tubu
mong these are rounded cells with oval nuclei
togonia. Transitional stages of the prima
lary spermatocytes are not uncommon T
and spermatozoa are visible in the course
n certain tubules.

Fig 2

Text Fig 2. Photograph of the bird after the plucking of
of feathers lateral view showing complete absence of the right

large intertubular spaces. The cell-outline is
and the nucleus is relatively large (nucleol

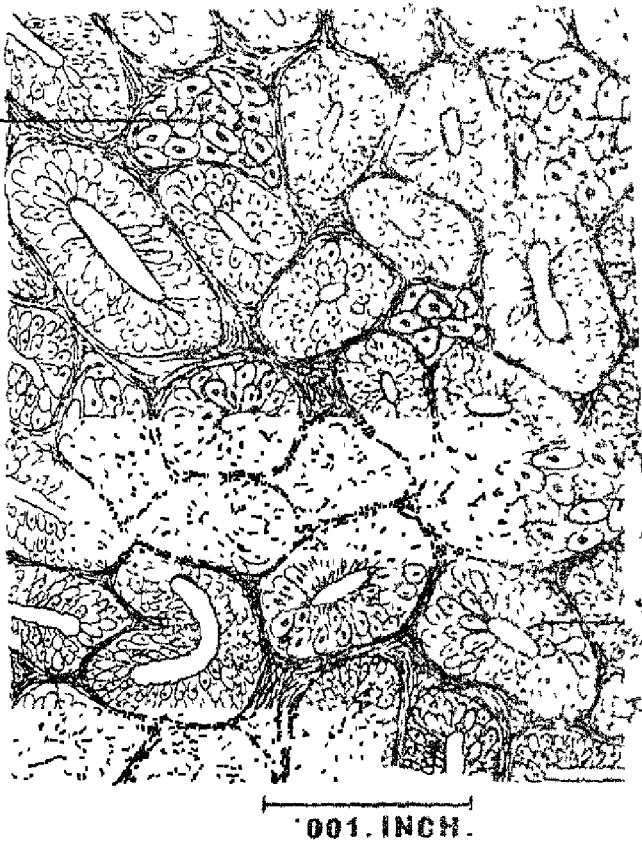


Fig. 3

Text Fig. 3. Transverse section of the testes show
large interstitial cells in the intertubular spaces.

IC—Interstitial cells in the intertubular spaces. S
thelium of the tubule

e cytoplasm is finely granular and contains n
d appears to contain little fat. In certain
vl, however, Prof. Morgan has shown that
ial tissue is very abundant in the testes and
ls become fat-laden. These fat-laden cells h
ntical with the so-called luteal cells (Pearl and
the ovarian type of interstitial cells (Fell. V

others) and are supposed to inhibit cock feathering. So far as the minute structure of the interstitial cells are concerned our observations would substantiate the views of Boring and Morgan whose researches have cleared up a very intricate problem in endocrinology.

THE ABNORMALITY OF THE HIND-LEG

We have not been able to find any recorded case of abnormality of the hind-leg of a fowl except the one recently published by one of us (G. N. R.) (16). We take the opportunity of describing another in this note.

Externally there is no indication of the existence of the right hind-leg (Fig. 2). The bird hopped with but one leg On dissecting the skin the peculiar arrangement of the muscles of the right leg showed clearly the defect to be congenital and not a case of amputation as we failed to discover any scar of healing. All the muscles were degenerate and lumped one above the other as shown in the figure 4 (MP).

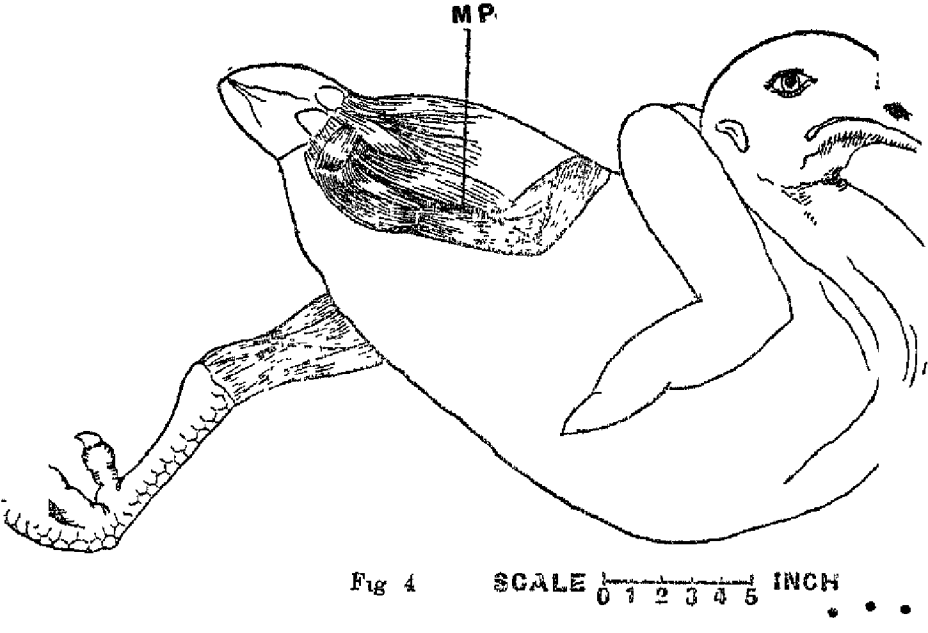
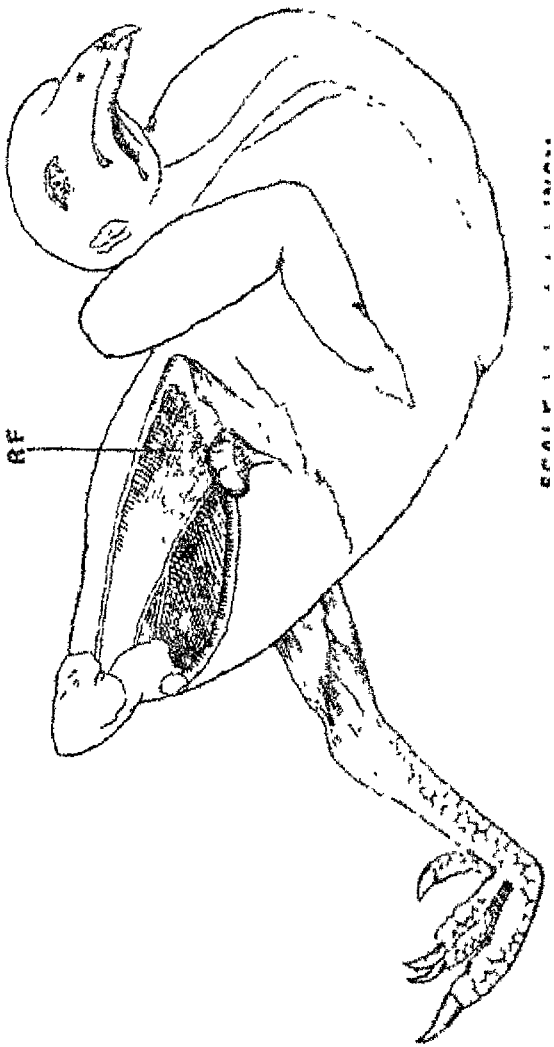


Fig 4

Text Fig 4. Dissection showing the arrangement of muscles of the right side in the pelvic region

MP—Muscles piled up one above the other in the pelvic region of the right side

On carefu y rem ving t c n us ics we can tiny nodule of bone about $\frac{3}{4}$th inch in length a in breadth attached to the acetabulum Fig. 5 RI the whole of the skeleton of the right hind-limb.



Text Fig. 5. Dissection to show the skeleton hind-leg—a small piece of bone attached to the acet.

RF—Skeleton of the right leg, the head is att acetabulum

The pelvic girdle is expanded and its bony parts are perfectly normal. The little nodular bone has got a distinct head like that of the femur which fits in the right acetabulum and forms a ball and socket joint.

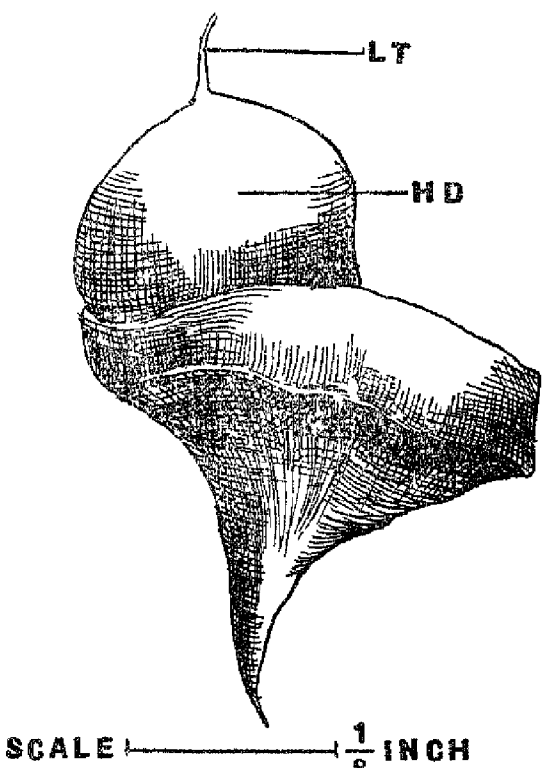


Fig 6

It has the usual binding ligament—the ligamentum teres (Fig. 6 LT) inserted from the head to the fundus acetabulum. The other ligaments, *viz.*, the capsular, which grasps the brim of the acetabulum and the head of the femur and the Pubo-femoral, binding the pubis and the femur, are wanting.

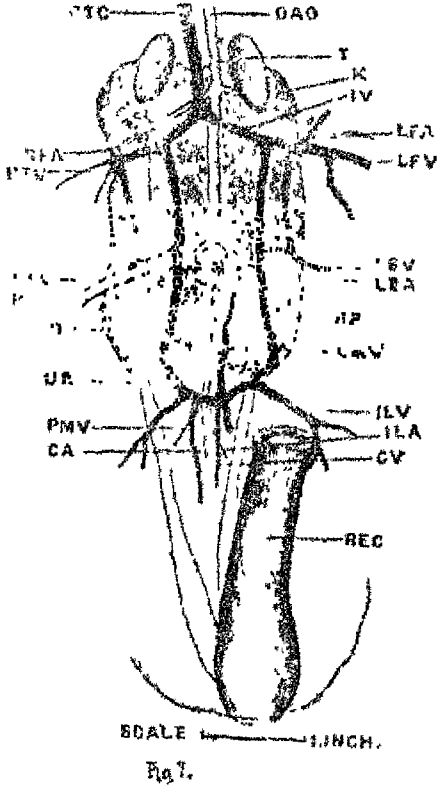


Fig. 7

Text Fig 7. Dissection of the arteries and veins of the pelvic region showing the narrow calibre of the right sciatic and femoral arteries and vein.

CA—Caudal artery. CMV—Coccygio-mesenteric vein. CV—Caudal vein. DAO—Dorsal aorta. IIA—Internal Iliac artery. IIV—Internal Iliac vein. IV. Iliac vein. K—Kidney. LFA—Left femoral artery. LFV—Left femoral vein. LSA Left sciatic artery, LSV—Left sciatic vein. PMV—Posterior mesenteric vein. PTC—Post Caval vein. REC—Rectum. RP—Renal Portal vein. RFA—Right femoral artery. RFV—Right femoral vein. RSA—Right sciatic artery RSV Right sciatic vein T Testes. UR—Ureter VD—Vas deferens.

The blood vessels in the pelvic region show normal structure on the left side while on the right they are markedly degenerate. The right femoral artery and vein (Fig. 7 RFA, RFV) are extremely narrow in calibre with a very few minute branches. The same is the case with the sciatic artery and the vein. These ramifications supply the degenerate muscles of the right leg (MP).

We wish to acknowledge here our thanks to Professor D. R. Bhattacharya for affording the necessary facilities for work and for offering helpful criticisms.

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ON TWO NEW SPECIES OF TREMATODES FROM ALLAHABAD

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INTRODUCTION

During the period from July to October 1930 a large number of tortoises and birds available at Allahabad were dissected by me. In this paper I describe two trematodes, one monostome of the family Notocotylidæ and the other distome belonging to the genus Astiotrema Looss of the family Lepodermatidæ Odhner.

The genus *Catatropis* Odhner is exclusively parasitic in the rectal cæca of birds. Hitherto four species of this genus have been recorded, two European species, namely, *C. verrucosa* and *C. charadrii*, Skrjabin, one North American species *C. fimbriata* and the last *C. gallinulae*, Johnston from Australia. The present paper contains one more species obtained from wild duck *Dafila acuta acuta* (pintail), a common bird of prey of Northern India.

Two species of the genus *Astiotrema* Looss from Indian fresh water tortoises have been recently described by Mehra. The present paper also contains an account of a new third species of this genus from a water tortoise *Emyda granosa* different from that of the other Indian species referred to above.

* The systematic position of the genus *Astiotrema* Looss has been fully discussed by Mehra in 1931 (4) I am

quite in agreement with the author that the sub-family *Astiotremanae*, Baer should be dropped and the genus be referred to the sub-family *Lepodermatinae* Looss. The work was carried out under the supervision of Dr. H. R. Mehra to whom I am greatly indebted for his help and guidance. I am also indebted to Mr. S. C. Verma, for his assistance.

ON A NEW SPECIES OF THE GENUS CATATROPIS ODHNER BELONGING TO THE FAMILY NOTOCOTYLIDAE

Catatropis orientalis sp. n.

Numerous specimens referable to a new species of *Catatropis* Odhner (Notocotylidæ) were found in the anterior quarter of the rectal cæca of *Dafila acuta acuta* (the pintail duck)—a common species of birds of the family Anatidæ available at Allahabad. Infection appears to be quite common. Almost every pintail dissected by me and Mr. S. C. Verma of the Zoology department was found to be infected by a large number of these parasites. The worms showed slow movements due to contraction and extension of the body. But these movements were not so marked as to cause the main excretory canals and the intestinal cæca to undergo similar contractions and extensions as in many distomes.

The worms while attached to the walls of the rectal cæcum have a crescent-shaped appearance, being strongly curved ventrad. In physiological salt solution they lived only for about three and half hours.

The body is thin and transparent. It is almost oval in shape, tapering more towards the anterior end than towards the posterior end which is always rounded. The length varies from 3·3 to 4·87 mm. and the maximum breadth, which lies in the anterior region of the testes from 1·18 to 1·5 mm The cuticle is devoid of spines or scales but in the anterior

region, up to the posterior margin of the cirrus sac it bears minute spiny indentations which hardly project from the surface. The ventral papillæ, which are broad at the base and more or less bluntly pointed at the free end, occupy the entire ventral surface of the body and are arranged in regular rows. Besides these papillæ there are present the ventral glands arranged in three longitudinal rows between the two intestinal cæca. The glands of the mid-ventral rows lie close to one another forming a continuous line extending from the anterior saccular part of the cirrus sac to about the posterior margin of the ovary. The other two rows, which are ventro-lateral in position are composed of a series of seven or eight groups of glands lying more or less separate from one another

The oral sucker is almost terminal, measuring 0·16 to 0·2 mm. in diameter A pharynx is absent The œsophagus is short, measuring 0·16 to 0·32 mm in maximum length. The intestinal cæca are almost of the same length; they extend nearly to the hinder end of the body exhibiting small diverticula throughout their length and terminating in a rosette-shaped blind end They lie about half way between the middle line and the body-wall, touching the outer limits of the uterine loops; but near the anterior region of the testes they curve inwards so as to occupy a position between the laterally situated testes and the median ovary.

The excretory system is typical of the genus. The excretory bladder is almost rounded, and opens to the exterior by a dorsally situated pore near the hinder extremity. The cavity of the excretory bladder is funnel-shaped and its inner wall is thrown into six distinct ridges which probably control the excretory opening, forming a structure called "rippon" by Looss. From the excretory bladder two main excretory canals are given off one on each side, which run forwards laterally almost parallel to and close outside the intestinal cæca These canals give off throughout their

course both external and internal short branches which in their turn further branch. The external ramifications extend to the lateral margins of the body. The main excretory canals run into each other by a transverse connection at the anterior end between the oral sucker and the intestinal bifurcation.

The testes are much lobed measuring 1·09 mm. in length and 0·49 mm. in breadth. They are longer than broad and are laterally situated close outside the intestinal cæca with their long axis straight and parallel to the length of the body, unlike the testes of *C. gallinulae* Johnston, which lie obliquely. The testes may extend anteriorly a little in the vitelline field. The vasa efferentia arise from about the middle of the inner margin of the testes The vas deferens was not observed on account of the massive vesicula seminalis and the uterine coils. The vesicula seminalis is coiled and lies outside the cirrus sac between it and the transverse uterine coils. The cirrus sac is median and more or less flask-shaped measuring 0·69 mm. in length and 0·21 mm. in breadth in the middle of its posterior half. The common genital opening is median and lies immediately behind the intestinal bifurcation.

The ovary is lobed and median situated in the intertesticular zone. It measures 0·33 to 0·49 mm. in length and 0·28 to 0·33 mm. in maximum breadth. The short wide oviduct arises from the anterior border of the ovary and takes a somewhat S-shaped course before it enters the slightly curved ootype which is surrounded by a fairly large mass of shell gland cells The shell gland mass measures 0·23 mm. in length and 0 28 to 0·34 mm. in breadth. The Laurer's canal arises from the ootype and opens to the exterior by a small median dorsal pore situated about the level of the posterior margin of the shell gland mass.

The first narrow part of the uterus forms a loop ventrally to the she g and mass and then continues into

series of about twenty transversely placed wide coils which occupy the entire intracæcal region from the anterior end of the testes to the posterior end of the cirrus sac. The uterine coils may overlap to a certain extent the intestinal cæca.

The vitellaria, 1·04 to 1·95 mm. in length, lie laterally in the middle third of the body, commencing 0·54 mm. distance behind the cirrus sac at about the level of the eighth uterine coil from the anterior end and terminating at the anterior margin of the testes or a little behind it. Each vitelline gland consists of twelve groups of two to four follicles each arranged in grape-like bunches. The transverse vitelline ducts arise near the hind end of the vitellaria; they pass between the uterine coils and the ovary and overlap the shell gland mass where they unite in the middle to form a conspicuous vitelline reservoir. The eggs measure 0·024 to 0·026 mm by 0·011 to 0·013 mm. in size and possess at each end a long polar filament which is thicker at the base.

The genus has been recorded for the first time in India. The present species resembles the Australian species *C. gallinulae*, Johnston, but differs remarkably from *C. verrucosa* commonly found in European birds, in the presence of polar filaments of the eggs. It also resembles *C. gallinulæ* in the size of its eggs, but it differs from it in the large size of the vesicula seminalis, large size of the testes, shape of the ovary, and size and shape of the body. The ovary in *C. gallinulæ* is rounded but it is lobed in *C. orientalis*. *C. orientalis*, however, resembles *C. verrucosa* and *C. charadrii* in its general shape and size of the body and in the number of uterine loops. But it differs from *C. charadrii* in the ratio of the length to the maximum breadth of the body which is 3 : 1 as compared to 4 : 1 in *C. charadrii*. It also differs from all the known species mentioned above in the arrangement and number of the ventral glands and papillæ.

TABLE 1

nowing the length of the body and of different organs.

Length of the body	Length of vitellaria	Length of testes	Length of ovary.	Length of cirrus sac
4 87 mm.	1 95 mm	0 9 mm	0 33 mm.	0 93 mm
3'6 mm	1'04 mm	1 mm.	0'43 mm.	0 69 mm.
3 34 mm.	1 14 mm.	0 87 mm.	0 42 mm	0 61 mm.
3 34 mm.	1 04 mm.	0 87 mm.	0'45 mm	0 6 mm
3'78 mm.	1 45 mm.	0 83 mm	0'49 mm.	0 61 mm
4 33 mm.	1'68 mm.	1'08 mm	0'45 mm	0'85 mm.
3 94 mm	1'29 mm.	1 09 mm.	0 43 mm.	0 65 mm.
3 76 mm.	1 54 mm.	1'08 mm	0'45 mm.	0 69 mm.
3 6 mm	1 24 mm	1 mm	0'45 mm.	0 64 mm.

Showing the breadth of the body and other organs.

Breadth of the body in the anterior region of the testes.	Breadth of the testes.	Breadth of the ovary.	Breadth of the cirrus sac.
1'5 mm	0 44 mm.	0 28 mm.	0'21 mm.
1'21 mm.	0'44 mm	0'29 mm	0 16 mm
1 32 mm	0'44 mm	0'28 mm	0 15 mm.
1'17 mm.	0 44 mm	0 28 mm.	0'16 mm.
1'32 mm.	0 48 mm.	0 33 mm	0 21 mm.
1 2 mm.	0'49 mm.	0'28 mm.	0'17 mm.
1 27 mm	0'43 mm	0'32 mm.	0 16 mm
1 28 mm	0 43 mm	0 28 mm	0 18 mm.

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ON A NEW SPECIES OF ASTIOTREMA LSS, ASTIOTREMA-GANGETICUS, WITH A KEY TO THE SPECIES OF THE GENUS

Astiotrema gangeticus sp. n.

Thirteen specimens of this species were obtained from the duodenum of *Emyda granosa*, the tortoises dissected at Allahabad Only one out of four was found to be infected with these parasites and a species of the genus *Cephalogonimus.*

The distomes were attached to the wall of the duodenum in the anterior one-third of its entire length. The parasites when kept alive in 0·75 per cent salt solution lived only for about forty-eight to fifty hours.

The body is almost oval or somewhat elliptical, measuring 4 to 6·7 mm. in length when flattened under the pressure of a cover-glass. The breadth varies in different regions as will be seen from the Table 2, but the maximum breadth lies at the anterior region of the anterior testis measuring 2·1 mm. Both anterior and posterior extremities are almost rounded, specially the latter, which is always broader. Spines are present only on the ventral surface, where they are arranged in regular transverse rows. The presence of spines only on the ventral surface is a characteristic feature of this species. The suckers are spherical and the ratio in their size is 4 · 5. The oral sucker lies at the anterior end or a little behind it, facing ventrally, and measures 0·18 to 0·28 mm in diameter The ventral sucker is median and

situated at about half way between th oral sucker and the middle of the body at about 0·69 to 1·3 mm. distance from the anterior end. It measures 0·26 to 0·37 mm. in diameter

The excretory bladder is Y-shaped The excretory opening is median, situated ventrally, a little in front of the posterior end of the body. The genital opening lies a little to the left immediately in front of the ventral sucker and much behind the intestinal bifurcation.

The mouth is terminal or slightly sub-terminal. A small pre-pharynx is present. The pharynx is almost pear-shaped measuring 0·16 mm. in length and 0·21 mm. in maximum breadth about the middle of its length. The œsophagus is a straight tube of moderate length and more or less uniform breadth, measuring 0 1 to 0·21 mm. in length. Only in one specimen it measured 0·51 mm. but the increase in length in this case may be due to much flattening on account of the excessive pressure before fixation. The intestinal bifurcation lies almost midway between the oral and ventral suckers at 0·25 mm. distance in front of the anterior margin of the acetabulum. The intestinal cæca are of unequal length, extending almost up to the posterior extremity, the right cæcum being slightly longer than the left one. The cæca are narrower in the anterior half of the body somewhat near the ovary behind which they gradually broaden attaining their maximum breadth of 0·1 mm. in the region between the anterior and posterior testes. Their course, for the greater part of their length, is almost straight , but they may have a slight bend towards the body-wall in the middle one-third of the body.

The ovary lies in front of the testes, to the right side touching externally or partly covering the right cæcum 0·16 mm distance behind the posterior margin of the ventral sucker. It is almost rounded with entire margin and measures 0·32 to 0·49 mm. in length and 0·37 mm. in maximum breadth in its middle region The shell gland comp ex

occupies nearly a median position at about the level of the inner margin of the posterior half of the ovary. The receptaculum seminalis is large and has more or less an elongated saccular form, measuring 0·92 mm. in length and 0·29 mm. in maximum breadth in its posterior half. In most cases it occupies an oblique position just behind the ovary covering dorsally the right intestinal cæcum and approaching the vitellarian follicles of that side. The Laurer's canal is a small narrow tube which runs parallel to the anterior half of the receptaculum seminalis and then bends outwards to open to the exterior slightly to the left of the mid-dorsal line a little behind the level of the shell gland mass.

The testes are much lobed. They are situated in the middle one-third of the body-length. In some specimens, however, the posterior testis may extend a little into the posterior third of the body. The anterior testis lies a little distance behind the ovary to the left side touching the inner wall of the left intestinal cæcum. It measures 0·58 to 0·93 mm. in length and 0·52 to 0·8 mm. in maximum breadth. The posterior testis is slightly larger showing the same range of variation in size as the anterior testis. The vasa efferentia arise from the middle of the anterior margin of the testes and unite to form the vas deferens at the level of the middle of the ovary. The vas deferens is of moderate length and runs parallel to the metraterm. The cirrus sac has thick muscular walls, measuring 1·06 mm. in length and 0·33 mm. in maximum breadth about the middle of its saccular part. It extends far behind the acetabulum, as far back as the middle of the ovary, with its long axis median, to the right or to the left side of the median line and parallel to the length of the body. Its narrow tubular terminal part lies dorsally to the right or left side of the ventral sucker except near the genital opening.

The uterus is much convoluted and both its ascending and descending parts pass between the testes forming a

large convoluted knot in the posterior third of the body. The metraterm runs about the median plane of the body inwards to the cirrus sac, passing dorsally to the acetabulum to open into the genital atrium. The eggs are oval in shape, measuring 0·042 mm. in length and 0·017 mm. in breadth.

The vitellaria lie outside the intestinal cæca, nearer them than the body-wall. The vitellaria commence behind the ventral sucker from about the beginning of the second quarter and terminate at about the end of the third quarter of the body. Each vitelline gland consists of a large number of follicles which lie close to one another in a continuous series, not in definite groups as in other species.

The species is characterised by the elliptical shape of the body, presence of spines on the ventral surface only, large size of the receptaculum seminalis, the ventral sucker being larger than the oral sucker, greater length of the intestinal cæca in proportion to the length of the body, the vitellarian follicles forming a more or less continuous chain and not divided up into separate grape-like bunches, large size of the cirrus sac, rounded form of the ovary, and the ventral subterminal position of the excretory opening. It, however, resembles *Ast. loossi* in the position and form of the testes, shape of the cirrus sac, the length of the œsophagus and intestinal cæca, elliptical form of the body and the ventral position of the excretory opening

KEY TO THE SPECIES OF THE GENUS ASTIOTREMA LSS.

The key to the species of the genus *Astiotrema* Lss. as given by Mehra is modified here in order to include the present species and the emended key is as follows :

Ovary obed *Ast loossi*

Ovary entire

(1) Intestinal bifurcation at posterior margin of ventral sucker ... *Ast. monticelli.*

(2) Intestinal bifurcation in front of the ventral sucker—

(*a*) Vitellaria terminating at middle of the anterior testis ... *Ast. inplectum.*

(*b*) Vitellaria terminating behind the anterior testis—

(i) Oral sucker slightly smaller than the ventral sucker ... *Ast. gangeticus Sp. n.*

(ii) Oral sucker slightly larger than the ventral sucker—

Diameter of suckers —0·25-0·3 mm.; testes broader than long—*Ast. reniformis*;

Diameter of suckers—0·36-0·62 mm: testes longer than broad.—*Ast. elongatum.*

TABLE 2

Showing the breadth of the body in different regions.

(A)

No of specimens	At the middle region	At the ant. margin of ant. testis.	At the ant. margin of acetabulum.	At the post. margin of the post testis.
1.	2 mm.	1·98 mm.	1·64 mm.	1·80 mm.
2	1·9 mm.	1·8 mm	1·49 mm.	1·87 mm
3.	1·9 mm	1·8 mm.	1·43 mm	1·78 mm.
4	1·74 mm.	1·73 mm.	1·54 mm	1·91 mm.
5	2·1 mm.	2 mm.	1·43 mm.	1·97 mm
6	2·08 mm.	2·08 mm.	1·69 mm.	1·96 mm.
7.	2 mm	2 mm.	1·63 mm.	1·92 mm.

(B)

the end itellaria.	At the ant. end near the ant. margin of the oral sucker.	At the post. end near the exc pore.
37 mm.	0·42 mm.	0 42 mm.
35 mm.	0 42 mm.	0·58 mm
08 mm.	0 53 mm	0·64 mm.
59 mm.	0 68 mm.	0·68 mm.
82 mm.	0 67 mm.	1·06 mm.
32 mm.	*0 19 mm.*	0·74 mm.
00 mm.	0 2 mm.	0·74 mm.

TABLE 3

ngth of the body, the length and breadth of eproductive and digestive organs.

Length of vitellaria	Length of œsophagus	Length of pharynx	Length of cirrus sac.	Length of ant. testis.
2 54 mm.	0·1 mm.	0 05 mm.	0 70 mm.	0 67 mm.
2·44 mm.	0·2 mm.	0 1 mm.	0·88 mm.	0 64 mm.
2 35 mm.	0·1 mm	0·1 mm	1 07 mm.	0·66 mm.
2 54 mm.	...	0·1 mm.	0·79 mm.	0·58 mm.
2 70 mm	...	..	...	0 69 mm.
3 65 mm.	...	...	...	0 93 mm.
3 61 mm				0 92 mm

No. of specimens	Length of post testis.	Length of ovary.	Breadth of the cirrus sac in the middle region	Breadth of ant. testis	Breadth of post. testis.	Breadth of ovary.
1.	0·64 mm	0·37 mm	0·23 mm.	0·58 mm.	0·64 mm.	0·29 mm
2	0·65 mm.	0·37 mm	0·32 mm	0·58 mm.	0·72 mm	0·29 mm.
3.	0·63 mm	0·32 mm	0·32 mm	0·68 mm	0·71 mm.	0·27 mm.
4.	0·90 mm	0·42 mm		0·85 mm.	0·74 mm	0·35 mm.
5	0·63 mm	0·32 mm.	0·32 mm	0·68 mm.	0·71 mm	0·35 mm.
6.	1·02 mm	0·49 mm	.	0·82 mm.	0·85 mm.	0·35 mm
7	0·95 mm	0·47 mm.	.	0·80 mm.	0·77 mm	0·37 mm.

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EXPLANATION OF FIGURES

Ventral View of *Astiotrema gangeticus*.

Transverse Section of *Ast. gangeticus* passing through the Region of the Ovary.

-Longitudinal horizontal Section of *Ast. gangeticus*

-Digrammatic Sketch of the Female Reproductive Organs as Constructed from the Series of Transverse Sections.

-Sketches Showing the Expansion of the Excretory Bladder in Living *Catatropis orientalis*.

Ventral View of *Catatropis orientalis* Showing the General Anatomy.

Digrammatic View of *C orientalis* Showing the Arrangement of the Ventral Glands.

Transverse Section of *C. orientalis* passing through the Region of Testes.

Digrammat c Sketch of the Female Reproductive Organs as Constructed from the Series of Transverse Sect ons

EXPLANATION OF LETTERING

Ant. Ts	...	anterior testis
c. s.	.	Cirrus sac.
ex. B.		Excretory bladder
ex. c.		excretory canal
ex p.	...	excretory pore.
G P	.	genital pore
Int c.	..	intestinal caecum.
L. c.		Laurer's canal
Me.	...	metraterm
Oes.		oesophagus
O S.	.	oral sucker.
Ov	..	ovary.
Ovi Dt.	...	oviduct
Ph.	..	pharynx
Post. Ts	.	posterior testis
R. S	..	receptaculum seminalis
S. G.	...	shell gland
Sp.	. .	ventral spines
Ts.		testis
Ut.	...	uterus.
Vas. df.	.	vas deferens
Vas ef.	...	vas efferens
V S.	...	ventral sucker.
Vt	.	vitellaria.
Vt. dt.	..	vitelline duct.
Vt. R.	...	vitelline reservoir.
V Sem.	...	vesicula seminalis.
Cu.	...	cuticle.
V. G	...	Ventral gland.

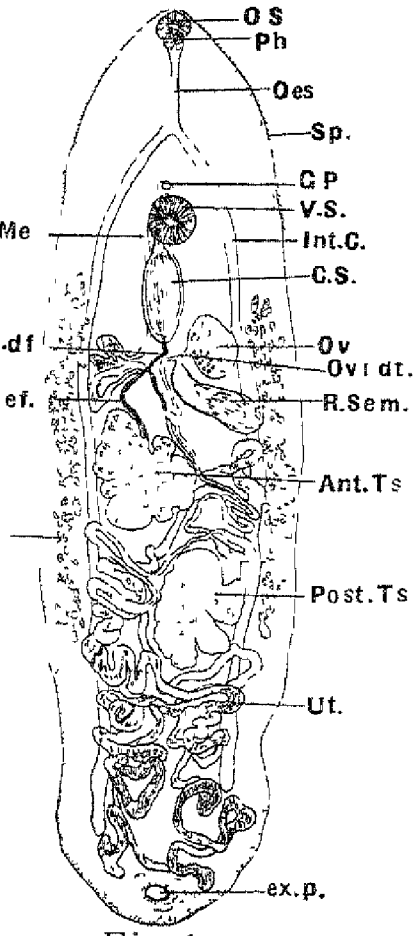


Fig 1

Dorsal View

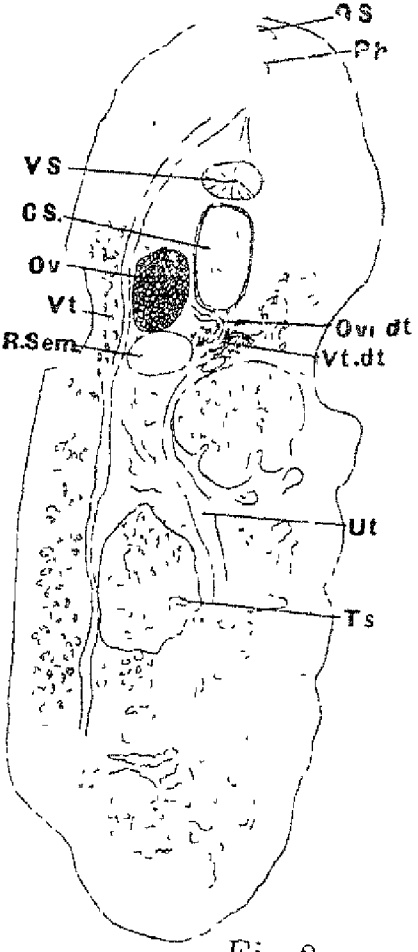
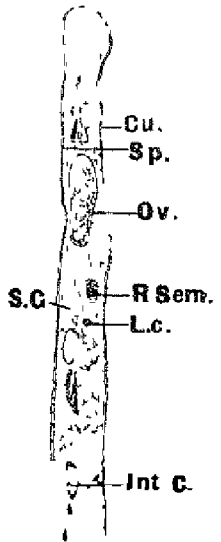


Fig 3

1 mm



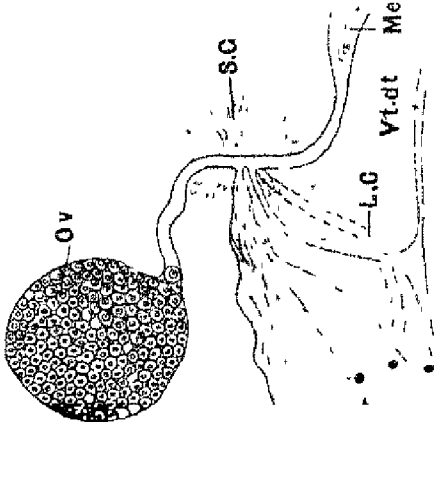


Fig 4

Plate 2

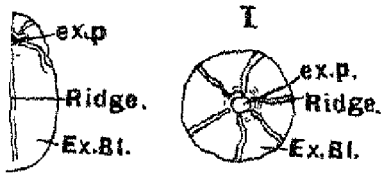


Fig. 5.

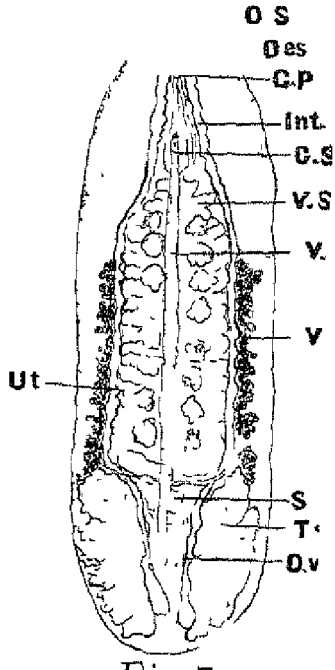


Fig. 7.

ex.c

Int. C.

Ut

Vt

S.C.

Ov.

x.p.

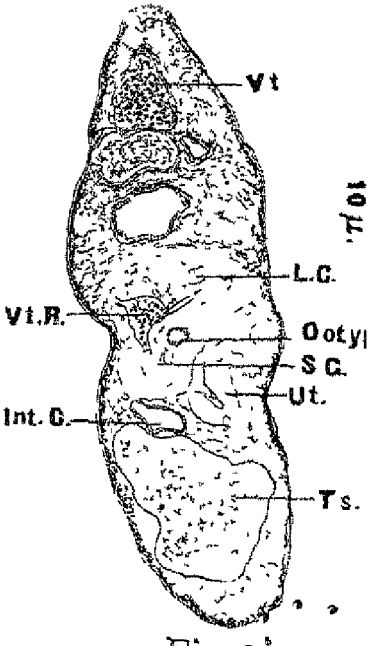


Fig. 8.

ON NEW DISTOMATE TREMATODES OF THE SUB-FAMILY TELORCHIINAE (FAMILY LEPODERMATIDAE) WITH A SYSTEMATIC DISCUSSION OF ITS GENERA

BY

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The distomes of the sub-family Telorchiinæ have received a considerable attention by the various workers on the group in Europe, America, and Australia but we have had no account of any Indian species till now. The present paper deals with a new genus *Paracercorchis* commonly found in a fresh water tortoise *Kachuga dhongoka* at Allahabad and a new species of the genus *Cercorchis* also met with in the same host.

Perkins in 1928 created two new genera, *Lecithopyge* and *Cercotecithos* for *Opisthioglyphe rastellum* Olsson and *Cercorchis arrectus* Molin respectively including them along with *Brachysaccus* and *Dolichosaccus* in the sub-family Telorchiinæ Travassos in 1930 has combined *Opisthioglyphe* and *Brachysaccus* in the genus *Opisthioglyphe*, and *Lecithopyge* and *Dolichosaccus* in the genus *Dolichosaccus*, thus reducing the number of these genera from four to two only While we agree with Travassos in assigning *Lecithopyge rastellum* to *Dolichosaccus*, we maintain that *Opisthioglyphe* and *Brachysaccus* should be recognised as separate genera The genus *Opisthioglyphe* which has

been included by some authors in the sub family Lepodermatinæ must be assigned to the sub-family Telorchiinæ as it closely resembles the genera *Brachysaccus* and *Dolichosaccus*—a fact which was pointed out before by Johnston (1912), Perkins (1928), and Travassos (1930).

Stunkard in 1916 combined the genera *Telorchis* and *Cercorchis* in one genus called *Telorchis*, but Perkins in 1928 following the previous workers, *i.e.*, Looss and Lühe separated and recognised them again as separate genera which he defined While we are in agreement with the latter author in this respect, we find that *Paracercorchis*[1] nov gen. combines in itself several important features of the genera *Cercorchis* and *Telorchis*, and also throws some light on the relationships of the genera *Protenes* and *Cercolecithos*.

The sub-family Telorchiinæ belongs no doubt to the family Lepodermatidæ as discussed by one of us in 1931 The Y-shaped excretory bladder with a long median stem, the cirrus sac with its contained organs, and the position of the ovary and that of the genital pore in the Telorchiinæ are very similar to those of the typical Lepodermatidæ, but the position of the testes behind the uterus and near the hinder end of the body sharply separates this sub-family from the other sub-families of the Lepodermatidæ. Though in *Telorchis* and some species of *Opisthioglyphe* and *Dolichosaccus*, the testes lie more forward midway between the genital aperture and the posterior end of the body, the uterus does not extend behind the testes to the hinder end as in the other sub-families of the Lepodermatidæ.

The cirrus sac and metraterm are exceedingly long and coiled in *Cercorchis dhongokii*—a feature which gives this species a unique distinction in the Telorchiinæ ; but as it resembles in almost all other points the other species of the genus *Cercorchis*, the great length of the cirrus sac

[1] We assign *Telorchis parma* (Braun) to *Paratelorchis* nov gen

and metraterm cannot be considered as characters of more than specific rank.

Paracercorchis Pellucidus nov. gen., nov. sp.

Out of thirty specimens of *Kachuga dhongoka* examined during 1930, only two were found to contain within the upper part of their small intestine forty mature specimens of this parasite. One immature specimen was also obtained in 1929 from the same host.

The distome is blackish in colour in the middle third of the body on account of the vitellaria and the innumerable eggs contained within the uterus, but the anterior and the posterior regions are greyish white. In entire mounts the specimens measure 7·12 mm. in length and 1·7 mm. in breadth in the region of the ovary and 1·4 mm. in that of the anterior testis. The anterior end is bluntly pointed and the posterior somewhat rounded. The anterior two-third of the body is covered with small backwardly pointed spines, which are numerous near the anterior end and which gradually decrease in number from before backwards till they disappear completely near the hinder end of the vitellaria The oral sucker is slightly larger than the ventral sucker measuring 0·28 mm. in diameter, but in the immature specimen it is double the size of the ventral sucker. The ventral sucker measures 0·27 mm. and is situated 2·0-2·6 mm. distance behind the anterior end, *i.e.*, at the end of the first one-quarter body-length. The genital opening lies slightly to the left, 0·44 mm. distance in front of the ventral sucker.

The pre-pharynx is absent, the pharynx is globular measuring 0 225 mm. in diameter. The œsophagus is short, 0·2 mm. in length and bifurcates almost behind the pharynx into the wider intestinal cæca which terminate a little in

front of the posterior end The cæca are somewhat swollen at their ends. Their wall is composed of a single layer of columnar epithelium surrounded by a layer of circular and longitudinal muscle fibres. In the immature specimen the pre-pharynx is absent and the œsophagus is longer than that in the mature specimens.

The testes lie in tandem near the posterior end of the body and have a deep notch at their posterior margin which gives them the appearance of a mammalian kidney. The posterior testis lies about 1 mm. distance in front of the hinder end and 0·1 mm. distance behind the anterior testis. Both testes are broader than long and of almost equal size measuring 0·8 mm. in breadth The vasa efferentia which could only be traced in sections arise as narrow ducts from the anterior surface of the testis. The vas deferens after entering the cirrus sac swells up to form a coiled thin-walled vesicula seminalis. The cirrus sac, 1 mm long and 0·3 mm. broad at its posterior end, is crescent-shaped and has thick walls composed of longitudinal muscle fibres. It is situated to the right side of the ventral sucker and partly overlaps it, extending behind as far as the ovary. The vesicula seminalis as usual is filled with sperms and occupies nearly one-third length of the cirrus sac. Its terminal tubular part which may be called the duct of the vesicula seminalis consists of two parts, a proximal narrow muscular tube of 0 05 mm. length and 0·007 mm. breadth, and distal broader part 0·22 mm. long and 0·022 mm. broad ; the latter is somewhat coiled and opens by a valvular opening into the pars-prostatica. This duct with its valvular opening probably controls the passage of sperms from the vesicula seminalis into the pars-prostatica. The pars-prostatica is tubular, measuring 0·35 mm. in length and 0·022 mm. in breadth, and is surrounded by the usual type of the prostate gland cells. The cirrus is small muscular and knob-like

The rounded ovary, 0·42 mm. in diameter is situated immediately behind the cirrus sac, usually touching the right intestinal cæcum, at 0·37 mm. distance behind the ventral sucker. A short narrow ciliated oviduct, 0·05 mm. in length and 0 012 mm. in breadth arises from the inner margin of the ovary near its posterior end and joins the small rather inconspicuous thick-walled duct of the receptaculum-seminis to form the ootype, where also the Laurer's canal joins from the opposite side. The receptaculum-seminis is thin-walled and rounded, measuring 0·12 mm. in diameter. It lies dorsally in the body and is always filled with sperms The Laurer's canal, 0·175 mm. long and 0·025 mm. broad, is a thick-walled ciliated duct which runs posteriorly to open to the exterior in the mid-dorsal line by a minute pore situated close behind the shell-gland-mass. Soon after the junction of the Laurer's canal with the oviduct the ootype turns ventrally to receive a small duct from the yolk reservoir and then becomes surrounded by the shell-gland-cells before passing into the uterus. The shell-gland-cells of the usual type are radially arranged around the ootype into which they open by their long narrow fibrillar ductules. The uterus arises as a narrow tube which turns towards the right side coiling spirally to form the right descending uterine coils. The descending uterus after reaching the anterior testis turns towards the left side to form the similarly coiled ascending uterus, which runs forward close inside the left intestinal cæcum. Its terminal end becomes less coiled and joins near the ventral sucker a short muscular metraterm of 0·8 mm. length, which opens into the common genital atrium in front of the opening of the cirrus sac.

The vitellaria lie laterally outside the intestinal cæca both commencing the same level, 0·12 mm. distance behind the ovary and terminating a short distance in front of the anterior testis, but not at the same level the left

gland being always longer terminates more posteriorly. Each yolk gland consists of a large number of follicles, arranged in grape-like bunches of twenty to thirty each. The lobes of the right, usually nine in number, are quite distinct, but those of the left one, about twelve in number, show a tendency to merge into each other. The longitudinal vitelline ducts lie in the narrow space between the intestinal cæca and the vitellaria and unite to form the transverse vitelline ducts in level with the second lobe of vitellaria. The transverse vitelline ducts run obliquely forwards towards the mid-ventral line and unite to form a yolk reservoir from which a common vitelline duct runs anteriorly to join the ootype in the shell-gland-mass.

The excretory bladder is typically Y-shaped. The long main stem bifurcates behind the ovary into the two cornua, which receive the common collecting ducts and their branches from the body on each side. The ova measure 0·0375 mm. by 0·0175 mm. in size.

SYSTEMATIC POSITION AND DIAGNOSIS OF PARACERCORCHIS

There is no doubt that *Paracercorchis* is closely related to *Cercorchis* and *Telorchis* which we recognise as separate genera, distinguished from each other by the size of the cirrus sac and the position of the genital pore, testes and the vitellaria. The genus *Paracercorchis* resembles *Cercorchis* in the vitelline glands restricted to the regions between the ventral sucker and the testes, larger number of follicle groups in the left vitelline gland, and the tandem position of the testes at the hinder end of the body but differs remarkably in the size of its cirrus sac and the metraterm (exceedingly long and coiled in *Cercorchis*), in the genital pore situated slightly to the left a short distance in front of the ventral sucker, and the vitellaria commencing behind the ovary and not in front of it or in

level with it as in *Cercorchis*. *Paracercorchis* differs from *Telorchis* in the position of the testes, which in the latter genus lie midway between the ventral sucker and the hinder end. In *Telorchis* the genital pore lies to the left side midway between the ventral sucker and body margin, and the vitellaria extend behind and over the testes. *Paracercorchis* resembles the genus Telorchis only in the relatively small size of its cirrus sac. It clearly follows from the foregoing points that *Paracercorchis* deserves the rank of a genus and though it combines in itself some of the characters of both the genera *Cercorchis* and *Telorchis*, it resembles the former more closely than the latter.

Diagnosis of the genus Paracercorchis.—With the characters of the sub-family. Body smooth or covered with spines. Suckers of about equal size. Genital aperture some distance in front of the ventral sucker slightly to the left side. Testes strictly in tandem, at the posterior end of the body, rounded or broader than long and kidney-shaped with a notch on their posterior margin. Cirrus sac short extending a little distance behind the ventral sucker and situated to the right side. Vesicula seminalis coiled and joined by a duct to the long pars-prostatica. Cirrus small and knob-like. Ovary rounded situated in the anterior half of the body close behind the cirrus sac to the right side. Receptaculum seminis and Laurer's canal present. Uterus inter-cæcal with descending and ascending uterine coils separated and regularly arranged in right and left halves of the body. Vitellaria laterally situated close outside the intestinal cæca, commencing behind the ovary and terminating a little in front of the testes.

REMARKS ON THE RELATIONSHIPS OF THE VARIOUS GENERA OF THE TELORCHIINAE

We give the following tree indicating the probable phylogeny of the genera of the sub-family Telorchiinæ. The

genus *Dolichosaccus* in which we include *Leecthopyge rastellum* occupies the base of this tree on account of a number of primitive features such as (i) the great variability in the position of the genital pore which though it lies close in front of the ventral sucker in *Dolichosaccus rastellus* has wandered away in the other species of the genus so as to lie close behind the intestinal bifurcation. The genital pore in the other genera has become fixed either close in front of the ventral sucker as in *Cercorchis* or more in front as in *Paracercorchis*, or still further forward as in *Brachysaccus* or forward and more lateral as in *Proteues*. (ii) The casual winding of the uterus into a few coils and its simple expansion during its course between the ovary and the testes in *Dolichosaccus* should also be regarded as a primitive feature. (iii) The enormous development of the vitellaria and the scattering of the vitelline follicles anywhere in the body that can provide sufficient space. (iv) Its habitat in the gut of Amphibian hosts and its distribution in Europe and Australia.

From *Dolichosaccus* we can derive the genera *Opisthoglyphe* and *Brachysaccus* which closely resemble it in many features. In *Opisthoglyphe* the cirrus sac has shifted forwards so as to lie close in front of the ventral sucker except in one species, *i.e.*, *Opisthoglyphe locellus* in which it occupies a primitive position adjacent to the ventral sucker. The genital pore has also shifted forwards and occupies a more or less varying position in the different species. The main stem of the excretory bladder is short and bifurcates behind the testes into the two cornua of about the same length as the main stem which we consider a secondary condition characteristic of this genus only.

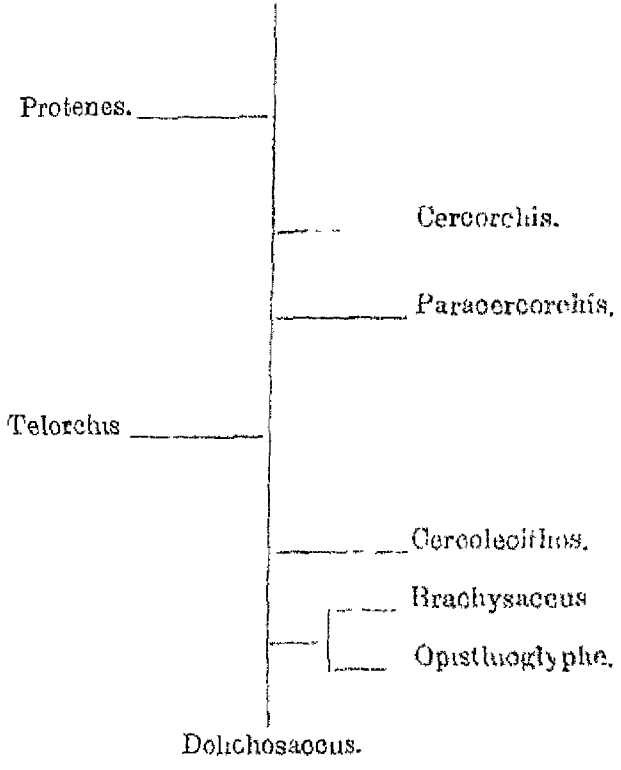
The genus *Brachysaccus* should be separated from *Opisthoglyphe*, in which Travassos has included it on account of the position of genital pore near the pharynx, shape

and position of the cirrus sac, the ovary being situated some distance behind the ventral sucker, the great development of the uterus, and the greater length of the main stem of the excretory bladder which bifurcates in front of the testes and not behind them. *Brachysaccus juvenilis* Nicoll, which resembles *Opisthioglyphe ranæ* in the general shape and relative position of its organs probably forms an intermediate species between the two genera.

We accept the genus *Cercolecithos* Perkins for the species *Cercorchis errectus* Molin. It appears that this genus occupies an intermediate position between *Dolichosaccus* and *Telorchis* on account of the great development of the vitellaria and their extension behind and over the testes and the coiling of the uterus into distinct ascending and descending tracts, but it is more primitive than *Telorchis* on account of the position of its genital pore, which lies median immediately in front of the ventral sucker.

As pointed out before the genus *Paracercorchis* stands between *Cercorchis* and *Telorchis*. *Protenes* should be considered as a specialised off-shoot from *Cercorchis* on account of the much forward and lateral position of its genital pore.

Tree showing the probable phylogeny of the various genera of the sub-family Telorchiinæ.



Cercorchis Dhongokii sp. nov.

Only once during 129 two mature specimens were obtained from the posterior part of the small intestine of *Kachuga dhongoka*. Of these we possess one in entire mount and the other in transverse sections.

The body is elongated and has a characteristic shape with the anterior extremity rounded and much broader than the posterior one. The length measures 11·3 mm. and the maximum breadth 1·7 mm. in the region of the ventral sucker. The breadth gradually decreases towards the posterior end, measuring 1·4 mm. in the region of the ovary and 1 mm. in that of the anterior testis. The anterior half of the body is covered with spines, which as usual are much more numerous near the ora sucker In the immature

specimens the spines are very small and hardly visible. The oral and ventral suckers are rounded and equal in size measuring 0·275 mm. in diameter. The ventral sucker is situated 1·2 mm. distance behind the anterior end. The genital pore lies immediately in front of the ventral sucker, *i.e.*, 0·03 mm. distance in front of it.

The prepharynx is absent. The pharynx is globular measuring 0·175 mm. in diameter. The œsophagus is absent, but in immature specimens it is fairly conspicuous. The intestinal cæca which arise directly from the pharynx on account of the absence of the œsophagus, occupy a lateral position near the body-wall extending almost to the posterior extremity.

The testes, somewhat rounded in shape, are situated close behind each other near the posterior end of the body. The anterior testis lies 1·2 mm. distance in front of the hinder end and is broader than long in slightly pressed specimens. It is slightly smaller than the posterior testis, measuring 0·58 mm. in length and 0·75 mm. in maximum breadth. The posterior testis measures 0·75 mm. in length and 0·69 mm. in maximum breadth.

The cirrus sac is enormously long, cylindrical and coiled in an open spiral, extending from the genital pore to the level of the ovary measuring 7·46 mm. in length and 0·22 mm. in breadth. It has thick walls composed of a thin layer of circular muscle fibres surrounded by a thick layer of longitudinal muscle fibres. The vesicula-seminalis is thin-walled and coiled occupying the basal portion of the cirrus sac of 0·56 mm. length. The pars-prostatica is long and tubular measuring 3·45 mm. in length and 0·1 mm. in breadth. It is also coiled due to the shape of the cirrus sac in which it is contained. Its wall is composed of an epithelial layer, the cells of which are greatly enlarged and vacuolated by the accumulation of the prostatic secretion in them. Around the epithelium there is a thin layer of

circular muscle fibres surrounded by a layer of longitudinal muscle fibres. The prostate gland cells form a large mass which almost fills the intervening space between the pars-prostatica and the cirrus sac. At about the middle of the length of the cirrus sac, the pars-prostatica passes into an extremely long and sinuous cirrus of 3·45 mm. length, *viz.*, about the same length as that of the former. The cirrus has thick muscular walls composed of an outer thick layer of longitudinal muscle fibres arranged in bands surrounding an inner layer of circular muscle fibres; it is entirely devoid of epithelium and is lined internally by the thick cuticle. In the retracted condition when it lies contained within the cirrus sac, it is produced into a number of narrow longitudinal outgrowths and is surrounded by a thick mass of fibrous parenchyma which fills the entirely intervening space between it and the cirrus sac.

The ovary is situated a little in front of the middle of the body to the right side in level with the basal end of the cirrus sac It is spherical, but in the flattened specimens it appears transversely elongated presenting an ovalish outline, measuring 0·29 mm. in length and 0·63 mm. in breadth. The oviduct is ciliated It arises from the middle of the posterior margin of the ovary, and after running dorsally towards the left for a short distance it turns towards the right side to join the Laurer's canal and the yolk reservoir The receptaculum seminis is very small rather rudimentary representing the internal end of the Laurer's canal. The Laurer's canal is slightly coiled and ciliated; it opens to the exterior dorsally in the region of the ovary by a small pore lined with cuticle. The ootype is surrounded by the radially arranged shell-glands of the usual shape. The uterus is much coiled, overlapping the intestinal caeca and consisting of the right descending and the left ascending parts which are not easily distinguishable. It joins the long muscular metraterm of 5 mm length and 0·225 mm breadth

at about the level of the junction of the pars-prostatica with the cirrus. The metraterm is coiled like the cirrus sac and is composed of a thick layer of longitudinal muscle fibres, surrounded by an equally thick layer of circular muscle fibres. The ova are small measuring 0·0375 mm. in length and 0·0175 mm. in breadth.

The vitellaria lie laterally near the body-wall overlapping dorsally and ventrally the intestinal cæca and the lateral extensions of the uterine coils They commence 1·8 mm. in front of the ovary, *i.e.*, about the middle of the distance between the latter and the ventral sucker, at about the junction of the uterus with the metraterm and terminate in front of the testes but not at the same level. The left yolk gland is longer and terminates always behind the right one. Each vitelline gland is composed of a large number of follicles arranged in lobes of 30—50 each, which nearly run into each other to give the gland a band-shaped appearance. There are twelve such lobes in the left gland and nine in the right one. The transverse vitelline ducts arise immediately behind the ovary and unite together to form a small vitelline reservoir which lies dorsally on the shell-gland-mass.

The excretory bladder is Y-shaped ; the long main stem bifurcates immediately behind the ovary into two cornua, which extend as far forwards as the ventral sucker. The excretory opening is situated at the hinder end of the body.

Diagnosis of C dhongokai.—Body elongated, spinous, broadest anteriorly. Genital aperture immediately in front of the ventral sucker. Suckers equal in size, ventral sucker situated at the end of the first one-sixth body-length Pre-pharynx and œsophagus absent ; intestinal cæca terminating near the hinder end. Testes strictly in tandem at the hinder end : anterior testis slightly smaller than the posterior Cirrus sac exceeding

long tubular and sinuous Metraterm sinuous and excee lingly long, nearly of the same length as the cirrus. Ovary situated to the right side in level with the basal end of the cirrus sac. Laurer's canal present; receptaculum seminis very small and rudimentary. Vitellaria laterally situated near the body-wall overlapping the intestinal caeca, commencing behind the ventral sucker at about the middle of the distance between the latter and the ovary, and terminating in front of the testes but not at the same level, the right gland ending in front of the left. Uterus extremely coiled overlapping the intestinal caeca. Excretory bladder Y-shaped, the main stem bifurcating immediately behind the ovary and the cornua extending as far as the ventral sucker.

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EXPLANATION OF PLATES I—III

Fig. 1.—Ventral view of adult *Paracercorchis pellucidus*.

Fig. 2.—Ventral view of a young *Paracercorchis pellucidus*.

Fig. 3.—Ventral view of an adult *Cercorchis dhongokai*.

Fig. 4.—Ventral view of a young *Cercorchis dhongokai*.

Fig. 5.—Diagrammatic view of male genetalia of *P. pellucidus*.

Fig. 6.—Diagrammatic view of female genetalia of *P. pellucidus*.

Fig. 7.—Diagrammatic view of female genetalia of *C. dhongokai*.

Fig. 8.—Transverse section passing through the region of Pars-prostatica of *C. dhongokai*.

Fig. 9.—Transverse section passing through the region of cirrus of *C. dhongokai*.

ABBREVIATIONS

A.T.	Anterior testis.	Ovd.	Oviduct.
C.	Cirrus.	P. T.	Posterior testis.
C. S.	Cirrus sac.	Par.	Pars-prostatica.
Com. Vit.	Common vitelline duct.	Ph.	Pharynx.
Cu.	Cuticle.	Pr. G.	Prostate gland.
Ex. B.	Excretory bladder.	Pro.	Prepharynx.
Ex. P.	Excretory pore.	R. S.	Receptaculum seminis.
G. P.	Genital pore.	S.	Spines.
In. C.	Intestinal cæcum.	Sh. G.	Shell gland.
L. C.	Laurer's canal.	U.	Uterus.
M.	Metraterm.	U. C.	Uterine coils.
O.	Ova.	V. S.	Ventral sucker.
œs.	œsophagus.	Ves. S.	Vesicula seminalis.
Or. S.	Oral sucker.	Vit. F.	Vitelline follicle.
Ot.	Ootype.	Y. R.	Yolk reservoir.
Ov.	Ovary.		

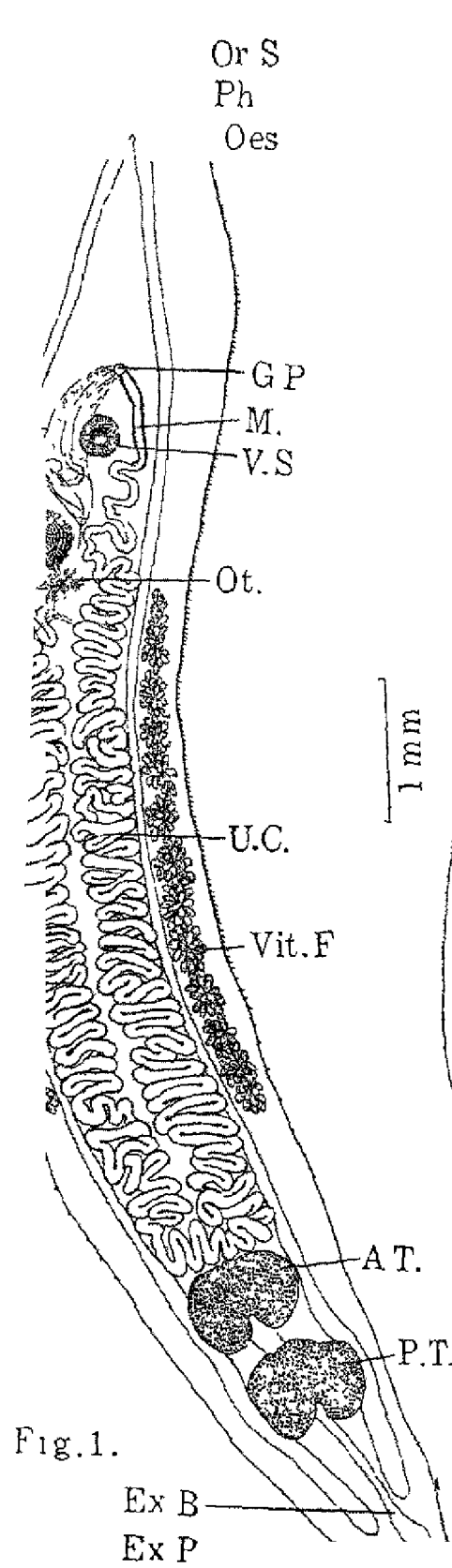


Fig.1.

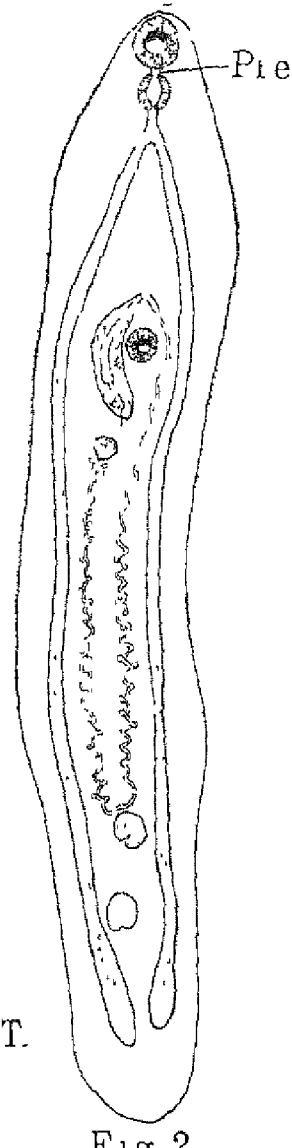


Fig.2.

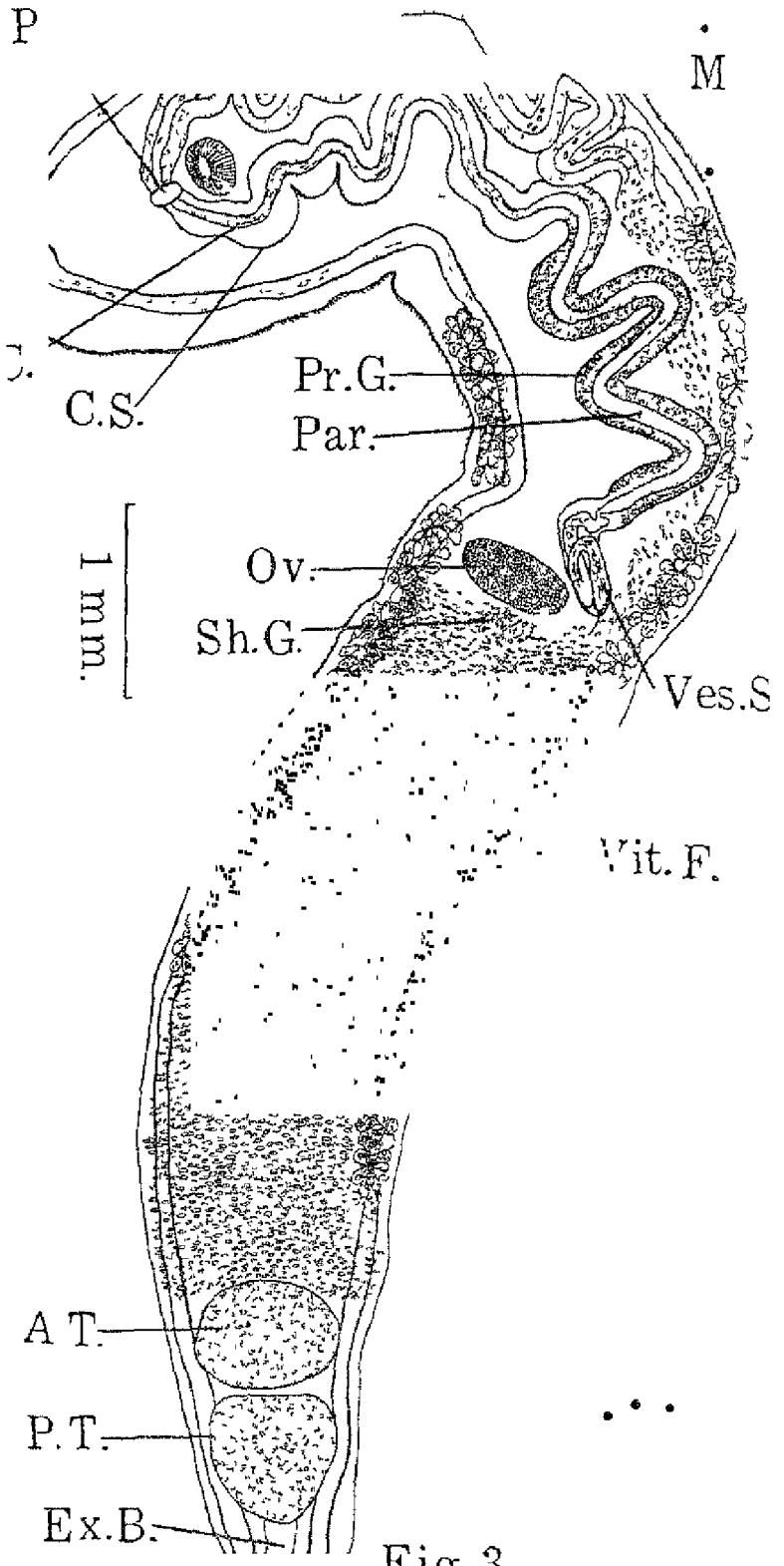


Fig 3

Plate 3

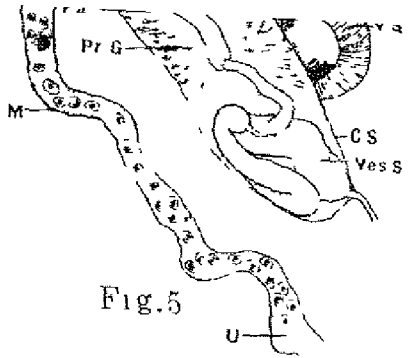


Fig.5

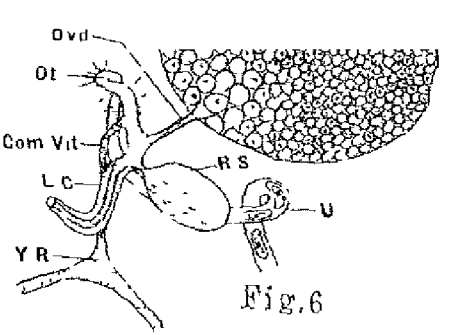


Fig.6

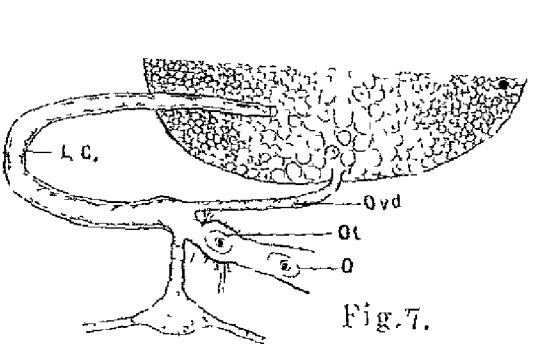


Fig.7.

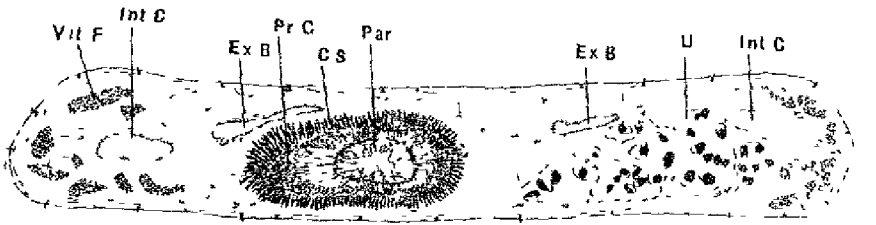
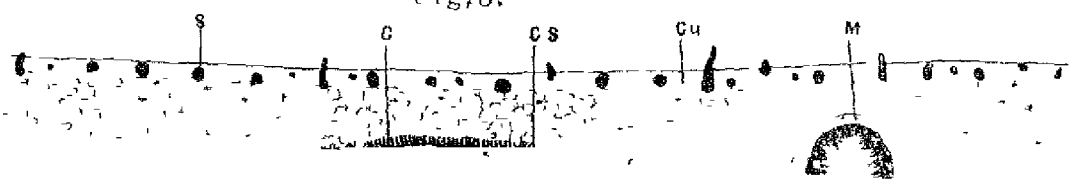


Fig.8.



ON CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF SCYLLA SERRATA (FORSK)

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INTRODUCTION

Recent work on the oogenesis of various animals has shown that the part played by the various cytoplasmic inclusions varies in nature considerably in different organisms. So far as the Crustacea are concerned, the only recent contributions are those of Harvey on the Plymouth shore crab, Carcinus maenas, and of King on a primitive Isopod, Oniscus. The Indian crustaceans have lain totally unexplored, and there is, therefore, enough justification for undertaking the present piece of work

In Oniscus, King (42) has found that the Golgi elements form the fatty yolk and that there are no nucleolar extrusions nor is there any area which may be considered to be homologous with the "Yolk nucleus of Balbiani." Proteid yolk, on the other hand, was observed to have been formed in relation to mitochondria. A peri-nuclear zone of mitochondria has been noted and after the formation of this zone the mitochondria are said to swell up and give rise to proteid yolk

Harvey (36) worked out the cytoplasmic inclusions with special reference to yolk formation in the oogenesis of Carcinus and has recorded interesting results. In the younger oocytes he could not discern the "Yolk nucleus of Balbiani" area. He has come to the conclusion that "fatty yolk is formed independently in the cytoplasm and albuminous yolk is produced in relation to Golgi bodies and probably also mitochondria."

The results noted above suggested a further examination with a view to throw more light on the method of yolk-formation in crabs. This work was also taken up by Nath and his collaborators at Lahore but due to paucity of material no conclusive results could be recorded.

MATERIAL AND TECHNIQUE

The specimens were collected near the delta of the river Ganges in Calcutta at fortnightly intervals from the month of September onwards. The female specimens collected for this work could easily be identified and distinguished from the male ones by the fact that they are of smaller size and possess a broad abdomen loosely attached on the ventral side of the thorax. The male specimens, on the other hand, are larger in size and each possesses a conical abdomen and clasping organs

In the month of September, female specimens of varying sizes were dissected but the ovary was so feebly developed in this season that even in the largest specimens nothing but a mass of germinal epithelial cells could be obtained in the form of a delicate transparent gland-like tissue. This tissue lying just beneath the dorsal carapace in the form of two coiled strings across the hepato-pancreas, dévelops gradually into a thicker ramifying mass in the specimens obtained during the months of October and November In the early period of the month of October

the ovary is fully packed with just differentiated oogonial stages and a few young oocytes By the end of the month of October and onwards till December we could find oocytes of various stages. Thus the most fruitful results were obtained from the experiments carried on from the month of October till the end of January. The ovary during this period begins to become mature and in larger specimens presents a fully matured reddish pulpy mass occupying a large area.

Small pieces of the ovary were fixed in various kinds of fixatives. The duration between the killing of the animal and the fixing of the ovary did not exceed a couple of minutes and thus all the possibilities of post-mortem changes in the tissue were minimised.

For the demonstration of the Golgi apparatus, the methods used were Da-Fano's Cobalt nitrate method, Cajal's uranium technique and Ludford's latest modification of the Mann-Kopsch fixative. The best results were, however, obtained by the techniques recommended by Da-Fano and Ludford.

Ludford's method proved to be most satisfactory for the demonstration of the Golgi apparatus. The material was fixed in Ludford's fluid (equal parts of saturated corrosive sublimate in salt solution and 1 per cent osmic acid) for eighteen hours and after being washed thoroughly for an hour with distilled water to remove every trace of corrosive sublimate, it was kept for three days in 2 per cent osmic acid at 35°-40°C. The sections were cut 5μ in thickness and the subsequent bleaching was effected by Henneguy's process, by treating the sections with 1 per cent aqueous solution of potassium permanganate for 5-10 seconds and then with 4 per cent solution of Oxalic acid for 1-2 minutes.

The slides were stained in Altmann's acid fuchsin, toluidene blue and aurantia.

In Da Fano's Cobalt nitrate method the material was fixed for 20 hours at 20° 25° C and then kept in a 2 per cent

solution of silver nitrate for 48 hours in order to effect proper impregnation. The extra silver was reduced by the methods indicated in the Vade-Mecum (27). Sections 6μ in thickness were cut and the slides were toned by 2 per cent gold chloride and 5 per cent hypo solution. The sections were stained either with safranine and light green or with iron alum and haematoxylin.

For mitochondria the best fixatives were found to be Champy-Nassonov's chrome-osmium technique, Regaud and Regaud-Tupa's bichromate methods. The material, after fixation in Champy's fluid, was put in 2 per cent osmic acid for post-osmication at 35°C for 5-7 days. Post-chromatisation was necessary in case of Regaud's fixative for 2-3 weeks.

All the osmic and dichromate preparations were stained by Champy-Kull method, *e.g.*, acid fuchsin, toluidene blue and aurantia.

For nucleolar extrusions, Bouin's picro-formol-acetic acid fixative was used which dissolves out cytoplasmic inclusions like Golgi bodies and mitochondria but fixes the nucleus and its derivatives. The sections were stained in Mann's methyl-blue-eosine and satisfactory results were obtained.

Pure turpentine free from all traces of acid was used in order to dissolve out all free fat if present. The proteid yolk bodies which appear as greyish brown bodies in osmic acid do not get dissolved in turpentine even after prolonged treatment; whereas, the fatty yolk bodies readily disappear being reduced by turpentine, leaving clear vacuolar areas each surrounded by an osmiophilic rim.

Centrifuge experiments were also carried out in a dark room during the winter months. The material was kept in the centrifuge which was rotated at the rate of 3,000 revolutions per minute. This operation was continued for three hours and the material was taken out speedily, and fixed as usual

INTRA-VITAM EXAMINATION

Intravitam examination of tissues has been tried by Parat, Gatenby, Mollendorff and others in order to demonstrate the disposition and behaviour of the various cytoplasmic inclusions in the fresh material. Various vital dyes, *e.g.*, Neutral red, Janus green B, Methylene blue, Nile blue, Trypan blue, etc., have been recommended to stain the various inclusions in the fresh material. Parat made use of Neutral red and Janus green B in very dilute concentrations to stain the Golgi bodies and mitochondria respectively.

The stock solution of the Neutral red and Janus green B was made according to Bhattacharya and Das's formula, *i.e.*, by dissolving ·1 gm. of fresh dye in 50 c.c. of 6/1000 salt solution. The stock solution in bottles was then placed in an incubator at 38°-40°C for 24 hours This solution is diluted to bring about a dilution of 1/25000.

Pieces of ovary were kept in this pink solution for about an hour and examined from time to time under oil immersion lens.

Besides these dyes 2 per cent osmic acid was also used as recommended by Gatenby, Bhattacharya, Nath and others for the examination of the yolk bodies. The fresh material was placed in 2 per cent osmic acid for about half an hour and then examined from time to time to note the effects of osmic acid on cell organs.

OBSERVATIONS

Golgi Apparatus

In Da-Fano preparations stained with Safranine and Light green, the Golgi elements appear as black bodies, some spherical in shape with a chromophilic rim and a chromophobic centre, and others representing a semilunar appearance—the Golgi crescents or dictyosomes In an advanced oogonium

(Fig 1 P 1) which has apparent y been differentiated out from one of the germinal epithelial cells, a few very small isolated black bodies (Gb) are visible in the clear cytoplasm. During this stage the cytoplasm presents a very clear and homogeneous area surrounding the nucleus (N), which itself is of a denser structure containing many nucleoli embedded in a sort of reticulum. For the sake of comparison and identification similar preparations of silver and osmic methods were examined and the results verified and confirmed. In the early oocytes the differentiation of the Golgi elements into a chromophilic rim and a chromophobic area is not well marked because of their being of extremely minute size; but in the older oocytes they appear as dictyosomes and vacuoles with a dark chromophilic rim and a clear chromophobic area. In the early stages the nucleus occupies the major portion of the oogonium surrounded by a thin area of marginal cytoplasm. There are many nucleoli within the nucleus at this stage and from the beginning they have a tendency to shift themselves to the periphery of the nucleus

Figs. 2, 3, 8, 9 (Pls. 1, 2) represent the early oocyte stages. Here the cytoplasm occupies a larger area surrounding the nucleus. The Golgi elements (Gb) increase in number and they lie closer to each other on one side of the nucleus. With the growth of the oocyte (Fig. 10, Pl. 2), the Golgi elements lying closer to each other accumulate in mass-formation in a juxta-nuclear position.

Figs. 4 and 11 (Pls. 1, 2, Y. Nuc.) show the formation of the area called the "Yolk-nucleus of Balbiani." The Golgi bodies (Gb) acquire the usual complex form as a compact massive structure situated adjacent to the nucleus, *i.e.*, in a juxta-nuclear position. The "Yolk-nucleus of Balbiani" has been described by Munson (50), D' Hollander (16) and others. This yolk-nucleus is the homologue of the archoplasmic area as recorded by Gatenby in his series of papers in the Q J M S and by Ludford 45) in Pate a It

is a focus of growth and dispersal so far as Golgi bodies are concerned and takes in stain readily, thus standing out in sharp contrast to the cytoplasm of the egg in general. At this stage we can notice easily the differentiation of Golgi elements into two kinds, (1) those that are spherical, (2) those that are crescent-shaped. Both possess a dark chromophilic rim and a clear chromophobic core. In close association with Golgi elements some bigger bodies are visible which are totally blackened with the reaction of the osmic acid probably due to their being fatty in nature. These bodies have been identified as fatty yolk bodies.

For some time this yolk-nucleus of Balbiani persists on one side of the nucleus but gradually the Golgi vesicles and dictyosomes begin to get detached from this compact mass and migrate into the general cytoplasm. (Figs. 5, 12, 13, Pls. 1, 2) The compact area can still be distinguished from the rest of the granular area (Fig. 13, Pl. 2). In a full-grown oocyte (Figs. 6, 14, Pls. 1 and 2), the yolk-nucleus is completely disorganised and the Golgi elements (Gb) are found scattered throughout the cytoplasm. The fatty yolk bodies (Fy) lying either independently or in close association with the Golgi elements are to be seen quite distinctly. In Ludford preparations some fairly large greyish brown spheres are visible. They are the albuminous yolk spheres (Ay). No apparent relationship has been observed between the Golgi elements and the formation of albuminous yolk.

Mitochondria

The best results were obtained from Regaud's formol-bichromate method followed by a prolonged chromatisation for 2-3 weeks, and from Champy-Nassonov's techniques. Mitochondria are also visible in Regaud-Tupa preparations. Dichromate techniques are exclusively meant for the demonstration of mitochondria whereas Champy's fluid may fix the Golgi elements and the associated yolk as we

In the oogonial stages the mitochondria granules or chondriome (M) (Fig. 15, Pl. 3) are observable in the form of a few dusty particles stained faintly with acid fuchsin. In the early oocytes (Figs. 16, 17, Pl. 3) the mitochondria come closer together adjacent to the nucleus and form, alike Golgi elements, a complex juxta-nuclear cap-like investment, the so-called "Yolk-nucleus of Balbiani"; (Y.-Nuc.) (Figs. 18 and 25, Pl. 3) This Yolk-nucleus area, in our opinion, functions as a centre of growth and dispersal for both Golgi bodies and mitochondria. This area may be regarded as the seat of intense cytoplasmic activity at a particular stage of development.

This heavily stained area gradually enlarges so as to surround the nucleus forming a perinuclear zone containing dusty mitochondria stained pink in acid fuchsin (Fig 21, Pl 3). Figs 23 and 27 (Pl. 3) represent fully developed oocytes where the mitochondrial granules have dispersed throughout the cytoplasm, and amongst these mitochondrial granules are found some big spheres stained cherry-red with acid fuchsin. These are the albuminous yolk bodies (Ay). These bodies are visible even in very early oocytes.

In spite of keeping the material for post-chromatisation after Regaud's bichromate technique (Bulliard's method—10a) for a period of more than a month, we observed no filamenter structures which could be identified as mitochondria.

It is a noteworthy fact that mitochondria remain very fine and dusty and the granules have not been observed at any stage to swell up or enlarge or take any part in the formation of yolk bodies.

Nucleolar Extrusions

The material was fixed in Bouin's picroformol-acetic acid fluid, so that the two important inclusions the mitochondria and Golgi bodies were dissolved out. The staining

was done by Mann s methyl blue eosin and very clear nucleolar extrusions were noticed lying outside the nucleus in a similar way as observed by Nath in Lithobius, Crossopriza, Scorpions, etc., Ludford in Patella, Gresson in Tenthredinidae, Harvey in Carcinus, and Gatenby in Saccocirrus.

Fig. 28 (Pl. 4) represents an early oocyte. The nucleus (N) is a large ovoid body occupying a large space in the oocyte, containing many small rounded nucleoli (Nu), basophilic in consistency (staining deep purple with Mann's methyl-blue-eosin and deep blue with haematoxylin). These nucleoli are embedded in the reticulum of the nucleolymph and the cytoplasm presents a clear, homogeneous area. In later stages (Figs. 29, 30, Pl. 4), one of the nucleoli grows in size and is converted into a prominent basophil nucleolus (B. Nu), whereas the other nucleoli have a tendency to move towards the periphery and plaster themselves around the nuclear membrane which looks like a beaded ring As the oocyte grows in size (Fig. 30, Pl. 4), the peripheral nucleoli have a tendency to come out of the nuclear membrane into the cytoplasm in the form of granular extrusions. In the meantime the basophil nucleolus becomes a very prominent body. It becomes larger and buds off deeply staining basophil bodies which pass out into the cytoplasm through the nuclear membrane (Figs. 29, 30, 31, Pl. 4). Figs. 29 and 30 represent oocytes in which a few granules budded off by the nucleolus, come out of the nuclear membrane, while others are still sticking to the membrane. Fig. 31 (Pl. 4) represents a later stage when the nucleolar extrusions have dispersed fairly evenly throughout the cytoplasm. It is to be noticed in these cases that the nucleolar emissions (N.E.) scattered in the cytoplasm are stained lightly with Mann's methyl-blue-eosin and are basophilic in the beginning but become acidophil bodies when scattered in the cytoplasm and ultimately disorganize due to fragmentation

This change in the behaviour of the nucleolar extrusions may be noticed easily by the staining reactions of Mann's methyl-blue-eosin. Probably the nucleolus during the period of its marked activity transforms itself into an amphophil body (A.Nu) containing round basophil bodies inside a lighter acidophil ground substance as observed by Nath in Buthus judaicus (57).

It is remarkable that the basophil nucleolus which persists even in older oocytes, occasionally, has a tendency to come out of the nuclear membrane as a whole into the cytoplasm (Fig. 37, Pl. 5). But, during this process no rupture of the nuclear wall has been observed. Probably in the cytoplasm also, it buds off some granular extrusions as observed by Nath (57) in Euscorpius napoli and Buthus judaicus. This shifting of the nucleolus as a whole from the nucleolymph to the cytoplasm (Figs. 37 and 38, Pl. 5) has been recorded by Nath (57) in scorpions and by Henneguy in vertebrates. These nucleolar extrusions have not been observed, however, at any stage to be directly metamorphosed into albuminous yolk spheres but probably they bear their influence in some way towards yolk-formation.

The Formation of Yolk Bodies

Two kinds of yolk bodies are easily distinguishable—the fatty yolk, and the albuminous yolk. The fatty yolk appears to arise through the intervention of Golgi bodies directly. In Da-Fano preparations there is very little possibility of the fat being fixed and the fatty yolk bodies appear as clear vacuolar spheres each in association with a Golgi element. Figs. 32, 33 and 34 (Pl. 4) represent oocytes at various stages of development showing the method of fatty yolk-formation. At the early stages of development inside and around the yolk nucleus (Fig 32 P 4)

some larger vacuolar bodies begin to appear amongst the scattered Golgi vesicles and dictyosomes.

In Ludford and Champy preparations also (Fig. 14, Pl. 2 and Fig. 35, Pl. 4) in close association with Golgi elements, larger spheres are visible which, being fatty in nature, appear as solid dull black bodies and do not show any sharply distinguishable osmiophilic rim or crescent. These spheres are fatty yolk bodies (Fy). To confirm their fatty nature, sections were treated with pure turpentine for varying periods and then examined under an oil-immersion lens. It was noticed that the original solid dull black bodies were totally decolourised leaving clear vacuolar areas either attached to an osmiophilic crescent or surrounded by a black rim, thereby proving their fatty constituency. The intermediate stages between the transforming Golgi bodies to fatty yolk spheres are also found. The Golgi elements which are non-fatty in the beginning, swell up and the fat is deposited within their chromophobic area (archoplasmic area). Thus there seems little doubt that these fatty yolk bodies are formed directly by the Golgi elements. In a well developed oocyte, these fatty yolk discs so formed by the metamorphosis of the Golgi elements are seen scattered throughout the cytoplasm.

Albuminous or proteid yolk has been described by different cytologists to have originated under the influence of either nucleolar extrusions or mitochondria, and sometimes, *de novo*, in the cytoplasm and rarely under the influence of Golgi bodies.

Nath (53) and Ludford (45) have observed in many invertebrates that nucleolar material from the nucleus comes out in the cytoplasm and contributes directly towards the formation of deutoplasmic inclusions or vitellogenesis. In early oocytes of Champy and Regaud preparations, onally we are able to notice some large bodies which appear as greyish brown spheres in osmic preparations and

stain cherry red with the Champy Kull method of staining Apparently, there exists no relationship between these yolk bodies and any of the cytoplasmic inclusions as detailed below. In the fully developed oocytes fixed in Champy-Nassonov, Regaud and Regaud-Tupa, individually, (Figs. 23 and 27, Pl. 3) we find a large number of proteid yolk spheres fairly large in size which take cherry-red colour with acid fuchsin, scattered throughout the cytoplasm. In the unstained preparation of Champy-Nassonov technique, these bodies appear as greyish brown bodies and even after prolonged treatment with pure turpentine these bodies have not been found to be in any way affected but remain as such throughout the development of the oocyte (A.Y., Fig. 35, Pl. 4) Their origin cannot be ascribed to any of cytoplasmic inclusions. Golgi elements cannot be said to have formed these yolk spheres as there exists no relationship between the Golgi elements and these yolk spheres. As regards mitochondria they have, at no stage of development, been observed to swell up to form these bodies. Mitochondrial granules always remain very minute, dusty and refringent bodies. Nucleolar extrusions also have never been observed to be directly metamorphosed into albuminous yolk spheres. Consequently these bodies may be looked upon to arise independently in the cytoplasm without any direct intervention of Golgi bodies or mitochondria or even nucleolar extrusions. This is how we conclude that these are albuminous or proteid yolk bodies as distinguished from the fatty yolk spheres.

Centrifuge Experiments

Of late the centrifuge experiments have proved immensely useful as they enable us to identify the cell organs in separate and distinct layers. Pieces of ovary were centrifuged for three hours at a speed of approximately 3,000 revolutions per minute and then quickly transferred

to the various fixatives. All the necessary precautions were taken to avoid any effects of disintegration in the tissue. All these operations were carried out during the winter months. Da-Fano and Champy-Nassonov techniques gave the most satisfactory results.

On examination of the material it was found that there were three separate and distinct zones formed in the mature oocytes. (Fig. 36, Pl. 5.) The lower zone is a pale brownish dense area fully packed with dusty and refringent mitochondrial granules (M) This zone is quite conspicuous and occupies about one-fifth of the whole oocyte The nucleus (N) lies in the middle with a large conspicuous oval plasmosome. The major portion of the middle area of the oocyte is fully packed with albuminous yolk bodies (A Y.), most of which have thrown out their contents during the revolution of the centrifuge machine and thereby appear as clear vacuoles. This zone occupies nearly the whole of the medullary region of the oocyte leaving on the upper extremity a small area consisting of dictyosomes and Golgi vesicles (Gb) associated with fatty yolk spheres (Fy). Discrete Golgi elements are also noticed to be scattered in the middle and lower zones individually as shown in the figure.

The disposition of the cytoplasmic inclusions and their formed components during the centrifuge experiments offer conclusive evidence as to the relations existing between them. It may be roughly concluded, therefore, that Golgi elements contribute to the formation of fatty yolk and that mitochondria do not take any part in vitellogenesis. Proteid yolk, on the other hand, arises *de novo* in the cytoplasm, may be, under the influence of nucleolar extrusions.

Vital-Colouration Experiments

The ovary was speedily taken out from the animal and transferred immediately into the physiological salt solution

Small pieces of the ovary were p aced in a trough contain ing a dilute pink so ution of Neutra red dye (1/2o 000) dilution for 20-45 minutes. The ovary was then teased out gently and examined from time to time in a dark room under oil-immersion lens in artificial light (1000 candle power). Some of the young oocytes (Fig. 39, Pl. 5), when examined carefully, were observed to contain a nucleus and a thick granular mass juxta-nuclear in position (Y. Nuc.) which we identify as the "Yolk-nucleus of Balbiani" which takes the same place in the fixed preparations topographically.

In more advanced oocytes (Figs. 40 and 41. Pl. 5), the Golgi bodies appear scattered in the general cytoplasm as discrete bodies, some vesicular with a chromophilic rim surrounding a chromophobic centre and others as crescent-shaped dictyosomes associated with an archoplasmic area. We get exactly similar bodies in fixed Da-Fano and Ludford preparations (Figs. 6 and 14, Pls. 1 and 2). In close association with these Golgi elements we find some large highly refractive vacuoles with a surrounding rim or a dictyosome. These are fatty yolk-spheres as ascertained by the treatment of the material with 2 per cent osmic acid.

Besides the above two types of structures, dispersed in between the Golgi elements, groups of very small vacuolar structures have been observed which take a cherry-red colour with the dilute pink solution of neutral red. These bodies make their appearance after the material has been in dilute neutral red dye for about 30-45 minutes. These have been identified as "Vacuome" by Gatenby (26), Bhattacharya (6), and Das (15) in other animals. There is apparently some relationship between the Golgi elements and the vacuome as it has been observed that round about patches of vacuome. some black dictyosomes or Go gi vesic es are situated (Figs. 40 and 41 P 5)

In a well-advanced oocyte (Fig 41, Pl. 5) (Vc) as many as four or five patches of "Vacuome" have been observed Nothing definite has yet been known regarding the function and behaviour of the "Vacuome" but it has to be admitted that it is a cell structure revealed only in fresh material. Fig. 42 represents an oocyte seen after treating the material with 2 per cent osmic acid for about 10 minutes and Fig. 43 represents another oocyte of about the same size examined after half an hour. The Golgi bodies appear as discrete heterogeneous elements, non-fatty in nature as proved by their remaining as refringent bodies (Gb). They are easily made out. In association with them some swollen Golgi bodies have been found, of a fatty nature, due to the deposition of free fat inside the chromophobic or archoplasmic area. Fig. 43 shows that the oocyte after having been treated for half an hour in 2 per cent osmic acid brings prominently into view swollen up Golgi elements which appear as darker spheres. The gradual stages between the developing Golgi elements and the formation of fatty yolk spheres are clearly noticed and it may evidently be concluded that the Golgi elements swell up and give rise to fatty yolk directly.

DISCUSSION

Golgi Apparatus

In considering the rôle of the Golgi apparatus in oogenesis we have to take into consideration all that has been discovered concerning its behaviour in the cell. In spite of the various differences in the form and behaviour of Golgi apparatus and mitochondria as described by Nath (55), Bhattacharya (4), Gatenby (21, 26), Parat (65, 67), Ludford (45), Weigl (78), and others, there are some common characteristics found in them. They are capable of independent movement within the cell They grow by assimilating the

necessary food substances from the cytoplasm and increase in number probably by fission

The exact behaviour of the Golgi apparatus as well as that of mitochondria during oogenesis, differs in detail in most cases that have been investigated. In the germ cells of the vertebrates and invertebrates the apparatus consists of separate rods, crescents, rings and sometimes granules. These are revealed by silver and osmic techniques. The differentiation of Golgi elements into a chromophilic rim and chromophobic area is almost common to all germ cells of vertebrates and invertebrates.

In the animal under investigation the best results to demonstrate Golgi apparatus were obtained by Ludford and Da-Fano techniques. The impregnation obtained in the case of Da-Fano after keeping the material in 2 per cent silver nitrate for 48 hours was specific, unlike the observation of Harvey who fixed the material for 4-6 hours only.

In a well-developed oocyte the Golgi elements exist in two forms. The spherical vesicular Golgi elements with a chromophilic rim and chromophobic centre and the semilunar forms or dictyosomes enclosing a portion of archoplasm.

Parat (65, 67) has recently emphasised that the Golgi elements and the vacuome are homologous. His conclusion is based on the assumption that the neutral red staining vacuome are Golgi bodies whereas the associated chromophilic substance is either an artifact or constitutes a special kind of mitochondria—the so-called Lepidosome.

Recently, Bowen (9) in plant cells, and Gatenby (25) in male germ cells have noticed the two above-mentioned structures lying separately. Gatenby observed that the so-called Golgi bodies of Parat are really vacuolar structures associated with crescent-shaped bodies—the dictyosomes. Gatenby, further describes the vacuome as an aggregation of vacuolar structures which are supposed to have been produced by the chromophilic rim of the Golgi elements So

the real substance of the Golgi element is constituted by the chromophilic rim (the dictyosome) and not by the associated vacuole—Parat's Golgi body.

In the youngest oocyte the Golgi apparatus lies in a diffused system consisting of a few granules which stain black with silver or osmic acid. The Golgi elements in a later stage form a compact mass, juxta-nuclear in position, the so-called "Yolk-nucleus of Balbiani." This structure has been described by various authors (50, 3, 15, 10) as the centre of growth and dispersal of Golgi elements. At this stage when the yolk-nucleus of Balbiani or the idiosome area (Bowen) is established, the Golgi bodies appear as discrete spherical and crescent-shaped dictyosomes.

Harvey in Carcinus (36), has failed to discern the formation of yolk-nucleus. He says, "Golgi elements increase in number eventually without any diminution in size and at this period a marked peripheral concentration of the Golgi elements becomes apparent." Again he adds, "As the yolk increases the yolk droplets occupy the outermost regions of the cell, until the majority of the Golgi elements are eventually crowded into the narrow perinuclear area . . . . ." In the animal under examination, no such perinuclear concentration of the Golgi bodies has been observed and also no relationship could be established between the yolk bodies (proteid yolk) and the Golgi elements. Golgi bodies have been observed to play an important part in the formation of fatty yolk, unlike the observation of Harvey (36) in Carcinus, where the fatty yolk is said to be formed from the cytoplasm independently and without the aid of any of the cytoplasmic inclusions. The formation of the proteid yolk by Golgi elements as observed by Harvey, in Carcinus, must be an interesting feature, because of its rare occurrence.

Mitochondria

In the oogonial stages mitochondrial granules are visible with great difficulty. In the early oocytes the mitochondrial granules, alike Golgi elements, occur in the 'Yolk-nucleus of Balbiani.' Harvey, in Carcinus, found "a slight concentration of mitochondria effected in immediate neighbourhood of nucleus," but he ascribes this concentration to the absence of a large number of mitochondria Later on, he observed a perinuclear zone of mitochondria. Probably Harvey's slight concentration of the mitochondria in the immediate neighbourhood of the nucleus is the "Yolk-nucleus of Balbiani" as described in this animal. Harvey might have missed the stages of the formation of the yolk-nucleus and therefore took into consideration only the perinuclear zone of mitochondria.

It is a remarkable fact that during the oogenesis of this animal the mitochondrial granules always remain dusty and granular. They have never been observed to increase much in size. In spite of very careful search these bodies have not been observed to take any part in the formation of any reserved food substances in the oocytes. Many authors have ascribed to mitochondria the formation of proteid yolk either directly or indirectly King, in Oniscus (42), records the proteid yolk as being directly formed by the swelling up of the mitochondrial granules. In Carcinus, Harvey has observed, "the albuminous yolk arises in the cytoplasm under the influence of Golgi bodies and probably mitochondria." But a careful search in this animal, has not revealed any relationship between mitochondria and albuminous yolk-formation.

During recent years many cytologists have been able to discover the filamenter mitochondria in the oocytes of many animals, *e.g.*, King (41). Hibbard (38). Das (15) Bullard (10a) and others In spite of post-chromatization

of the material for more than six weeks no filamenter mitochondria could be observed in our material.

We are inclined thus to conclude that mitochondria plays a rather insignificant part in the oogenesis of this animal.

Nucleolar Extrusions

Recently Harvey in Carcinus (36), has observed a process of nucleolar budding and the "probable emissions of nucleolar substance" from the nucleus to the cytoplasm. We have tried to substantiate the above conclusion by carefully working out the nucleolar behaviour during the oogenesis of this animal. Alike the observations of Harvey, we find, there are many nucleoli in the beginning but in later stages of development one nucleolus becomes prominent and gives out the extrusions. In this animal it has been definitely observed that the nucleolus in the beginning is a basophilic structure which afterwards turns oxyphilic.

The change in the staining reactions of the basophilic nucleolus into oxyphilic bodies in the cytoplasm has been observed by Nath in Culex (59), and more recently in Spider (58), and Scorpions (57). In Euscorpius napoli and Buthus judaicus, there is copious discharge of prominent round and deeply staining basophil bodies from the nucleus into the cytoplasm of the egg. "They are first basophil and later become acidophil and ultimately disappear as whole bodies." Gresson (31, 32), working on the oogenesis of sawflies (Tenthredinidae), has observed that in the early oocytes of Thrinax macula, the nucleoli are basophilic. As the oocytes increase in size the nucleoli develop an oxyphilic margin, which later on become rounded off and separate from the basophilic body. The basophil nucleolus buds off a number of basophilic extrusions which remain embedded in the nucleolymph and have not been observed to pass out in the cytoplasm. The oxyphilic part in

the meantime undergoes a period of activity and numerous oxyphil buds are liberated which migrate towards the nuclear membrane and eventually pass out into the cytoplasm.

Ludford in Patella (45, 46), has also observed a remarkable differentiation of the nucleolus into an oxyphil and basophil part. He suggests that the oxyphil nucleolus of the early oocytes gives rise to a basophil portion and then they gradually separate till both of them bud off extrusions of both kinds. But in ooplasm only oxyphil bodies have been observed whereas the basophil ones remain within the nucleus.

Wilson points out that the staining reactions of the nucleoli often vary materially at different periods in the history of the nucleus so that the same nucleolus may be at one time oxyphilic and at another time basophilic.

In our material it has been observed that the staining reactions of the nucleolus and nucleolar extrusions change from basophilic to oxyphilic during their passage from the nucleus to the cytoplasm. Occasionally, it has been observed in this animal, that the nucleolus as a whole or a major part of it comes out from the nucleus to the cytoplasm apparently without injuring the nuclear wall. This is, no doubt, an interesting phenomenon and has also been observed by Nath (57) in Scorpions. No sooner, it lies in the ooplasm, than the staining reactions are reversed and an oxyphilic structure instead of a basophilic one is noticed. Frequently, this oxyphil body seems to bud off oxyphil extrusions in the cytoplasm.

Bhattacharya (3), Nath (53), Gatenby (22), Ludford (46) and others working on vertebrates and invertebrates have laid stress upon the phenomena of nucleolar extrusions and in certain cases have attributed to the nucleolar extrusions the origin of albuminous yolk. It may, therefore, be said with a fair amount of certainty, that in many animals the nucleolar extrusions take part in the formation of proteid yolk either directly or indirectly

In this animal no direct metamorphosis of the extrusions into albuminous yolk has been observed.

Yolk Bodies

During the last few years, opinion seems to be crystallizing on the fact that there are two types of yolk bodies, (i) Fatty yolk, and (ii) Albuminous or Proteid yolk. The origin of these yolk bodies has been a subject of much controversy among recent workers in Cytology and the views upheld by various authors are sometimes contradictory. Some ascribe the origin of fatty yolk to *de novo* formation in the cytoplasm There are a few who ascribe the formation of fatty yolk to the metamorphosis of mitochondria but most of the modern cytologists agree that fatty yolk arises directly or indirectly in relation to Golgi elements. Nath (53), Gatenby (28, 29), Ludford (45), Bhattacharya (3), Das (15), and several others uphold this view.

Gatenby and Woodger (28), Ludford (45), and Brambell (10), showed that in Helix, Limnaea, and Patella, the fatty yolk is formed directly by the Golgi elements. Hirschler has similarly shown that in Ascidians (Ciona), the Golgi elements are directly metamorphosed into fatty yolk. The senior author (3, 4, 5) and his collaborators, have in a number of vertebrates, proved the direct or indirect transformation of Golgi elements into fatty yolk.

Nath, in a series of papers (53), has strongly emphasised the fact that Golgi bodies give rise to fatty yolk. In certain cases, the non-fatty chromophobic area or the vacuolar area of the Golgi vesicles is directly transformed into vacuolar fatty yolk bodies in the course of development of the oocytes (Spider, Scolopendra, Cockroach, etc.); in others, they are from the very beginning fatty in nature (Luciola, Dysdercus), and grow in size to form big yolk bodies This fatty yolk is dissolved out when treated with turpentine caving osmiophilic rims and crescents round

Gatenby (22) and Ludford (45) in Saccocirrus and Patella respectively have shown that the fatty yolk arises by the swelling up of the Golgi bodies.

Recently Hibbard (38), and Harvey (36), have claimed that fat arises independently in the cytoplasm without any relation to Golgi bodies and mitochondria in the eggs of Discoglossus and Carcinus respectively. Harvey in Carcinus observed that there was no relationship between the Golgi bodies and the fatty yolk In the animal examined by us, the fatty yolk has been observed to be formed directly by the Golgi elements.

In Da-Fano preparations, the fatty yolk is early represented either by an archoplasmic area to which a dictyosome is attached or a vacuolar area surrounded by a chromophilic rim. In the Champy technique, fatty yolk bodies appear as solid dull black bodies. The black bodies after treatment with turpentine are readily differentiated leaving clear vacuoles with an osmiophilic rim or a crescent. Intermediate stages between the fatty yolk bodies and the growing Golgi elements have also been observed. Thus it is concluded, that the fatty yolk bodies are formed directly by the swelling up of the Golgi elements. Most probably, as Nath conjectured, Harvey has been dealing with fat droplets and not fatty yolk bodies.

The Albuminous yolk has been observed even in very young oocytes. They do not seem to possess any relationship with the Golgi bodies and mitochondria and probably arise *de novo* in the cytoplasm.

Parat and Hibbard have demonstrated in several animals (Perca, Discoglossus, Aplysia, etc.), the relation between proteid yolk-formation and Golgi bodies. Similarly, Weiner in Lithobius and Tegenaria has shown that proteid yolk is formed on the periphery of the egg, among and in intimate relation to Golgi bodies. There are others, *e.g.*, King in Oniscus Gatenby and Woodger in Apantales,

who attribute the formation of proteid yolk in relation to mitochondria.

In quite a large number of animals (Invertebrates) the work carried on in this line has shown that proteid yolk is formed mostly in relation to nucleolar extrusions. Nath in a series of animals (Luciola, Lithobius, Spider, Cockroach, Scorpion, Dysdercus, etc.) has observed remarkable nucleolar extrusions given out by the nucleolus, which pass out to the cytoplasm and are either directly or indirectly transformed into proteid yolk.

Harvey has observed proteid yolk-formation in relation to Golgi bodies and further says, "probably it is deposited in the chromophobic part thereof." The present authors have been unable to find any existing relationships whatsoever, between the formation of proteid yolk and the Golgi elements or mitochondria in the animal under discussion.

Moreover, in spite of the fact that nucleolar extrusions are present in the cytoplasm, they have never been noticed to give rise to proteid yolk bodies directly. Thus it is assumed that the proteid yolk spheres are formed *de novo* in the cytoplasm.

The centrifuge experiments also confirm the above conclusions as we notice that neither the mitochondria nor the Golgi elements have any direct relationship with proteid yolk-formation whereas the Golgi elements are in close association with the fatty yolk bodies. Thus, the conclusion is forced on us that fatty yolk is formed directly by Golgi elements while the albuminous yolk is produced *de novo* in the cytoplasm.

Vital Colouration Experiments

Since the vital staining methods offer satisfactory results in this animal, it is worthwhile discussing in this paper, briefly, the supposed homology of the Golgi bodies and vacuole

Parat (65 67 69) with his collaborators for the first time demonstrated the occurrence of vacuome in the animal cells by vital colouration methods and believed that the Golgi apparatus and the vacuome were homologous structures. Further, the examination of salivary glands, pancreas, etc., led Parat to the conclusion that the Golgi apparatus is constituted of a system of vacuoles (Vacuome) in which "Granules de secretion" are produced by a process of condensation. He observed that the Golgi bodies are really the vacuoles which are stained with dilute neutral red, and that the osmiophilic rim or crescent is an artefact or is constituted of some special kind of chondriosome, lipoidal in nature, which is associated with the vacuole occasionally. To these special chondriosomes he gives the name of "Lepidosomes." Thus, according to this view, the vacuolar space represents the vacuome ("Golgi body"), which may be surrounded by special chondriosomes called Lepidosomes.

Recently, Bowen (9) in plant cells, and Gatenby (25) in male germ cells of animals, have vehemently criticised Parat's Lepidosome theory. Gatenby, taking into consideration the definition of the Golgi apparatus maintains that "It is an argentophil structure discovered in the nerve cells as such by Golgi" (26). He further adds that the so-called Parat's "Lepidosome" is the real Golgi element associated with an archoplasmic area or the vacuole. In the male germ cells some of the vacuoles are secreted by the Golgi elements and they collectively form congeries of vacuoles—the "Vacuome," staining with the dilute neutral red solution. Thus he contradicted the view held by Parat that Golgi bodies are vacuoles whereas the argentophil structure is an artefact.

Very recently, Beams and Goldsmith (1), in the salivary glands of Chironomus larva, observed that probably the "neutral red bodies are in reality the secretory inclusions which have been coloured by the dye." They conclude that the neutral red bodies cannot represent the Golgi

bodies, the latter being argentophil in structure and are never found to be coloured with neutral red. Bhattacharya and Das in the ovary of the young pigeons (6), have found that Vacuome is quite a different structure and cannot be confused with the discrete Golgi elements as both these structures can be seen at the same time in vital examination lying separately as distinct structures. There appears to be, however, a close relationship between this "Vacuome" and the Golgi crescents, the latter being sometimes associated with the former.

Nothing definite can be said yet as to the behaviour and function of these neutral red staining bodies.

A TABULAR REPRESENTATION OF THE VARIOUS CYTOPLASMIC INCLUSIONS AND THEIR RELATIONS IN REGARD TO VITELLOGENESIS IN SCYLLA SERRATA.

Oogonial stages	Early oocytes.	Fully developed oocytes.
Golgi Bodies—		
Form.—Granular	(1) Vesicular - chromophilic rim and chromophobic centre (2) Crescent-shaped (dictyosomes).	Same as in early oocytes
Disposition.—A few in number, adjacent to the nucleus.	Formation of the "Yolk nucleus of Balbiani."	Scattered in the cytoplasm
Function:—Nil.	Swollen up Golgi elements with one or two fatty yolk bodies.	Many fatty yolk spheres.
Mitochondria—		
Form:—Granular and dusty	Granular and dusty	Granular and dusty
Disposition:—Few, adjacent to the nucleus.	Formation of "Yolk nucleus of Balbiani."	Scattered throughout.
Function.—Apparently nil	Apparently nil	Apparently nil

Oogonial stages.	Early oocytes.	Fully developed oocytes .
Nucleolus—		
Form —round small bodies	Large, oval.	Large, oval.
Disposition .—Many nucleoli embedded in the nucleolymph, tendency to arrange themselves around the nuclear membrane.	One becomes prominent. Others plaster themselves round the periphery of the nucleus.	Basophil nucleolus gives out nucleolar extrusions which become oxyphil in the ooplasm.
Function :—Apparently nil, but may influence the indirect synthesis of the protein Yolk.		. .
Fatty Yolk :—None	Few	Numerous
Alb. Yolk. None	A fair number	Numerous.

A TABULAR REPRESENTATION OF YOLK FORMATION IN ANIMALS

Genus or animal	Author	Fatty yolk	Albuminous yolk.	Nucleolar extrusions, if present.
Grantia	Gatenby, J. B.	. .	In ground protoplasm	...
Ascaris ...	Hirschler . .	Cytoplasm .	Mitochondria	..
Saccocirrus	Gatenby	Golgi bodies	Nucleolar extrusions.	Yes.
Peripatus.	King ..	Formed in groups but its source has not been determined	...	Probable.

Genus or animal	Author.	Fatty yolk	Albuminous yolk	Nucleolar extrusions, if present.
Carcinus ...	Harvey ...	Cytoplasm (independently).	Golgi bodies.	Probable
Oniscus ...	King ...	Golgi bodies	Mitochondria	...
Limulus . .	Gardiner ...	Nucleolar extrusions.	Interaction of Golgi bodies, mitochondria, and nucleolar extrusions.	Present
Palamnaeus	Nath ..	Golgi bodies	None ...	None
Scorpion	Nath ...	Do. ...	Nucleolar extrusions.	Yes
Cockroach	Nath and Piere Mohan.	Do ...	Do ...	Do
Scolopendra	Nath and Hussain.	Do ..	Do ...	Do.
Luciola ...	Nath and Mehta.	Do ..	Do. . .	Do.
Lithobius ...	King ...	Probabaly Golgi bodies	Do. ...	Do.
,,	Nath ..	Apparently Golgi bodies.	Do. ...	Do.
,,	Weiner ..	Cytoplasm ...	Indirectly from Golgi bodies.	...
Tegenaria	Weiner ...	Vitelline layer	Golgi bodies	...
Spider (Crossopriza)	Nath	Golgi bodies	*De novo* in the cytoplasm.	None

Genus or animal.	Author.	Fatty yolk.	Albuminous yolk.	Nucleolar extrusions, if present
Culex ...	Nath ...	Fat deposits in microspheres.	Microspheres or proteid yolk bodies.	...
Apantales	Gatenby	...	Mitochondria and secondary nuclei.	...
Tenthredinidæ	Gresson ...	Golgi bodies	Nucleolar extrusions.	Yes.
Daphnia ...	Hill and Gatenby	Golgi bodies	None ...	None.
Nepa .	Steopoe ...	.	Golgi bodies	...
Dysdercus	Nath . .	Golgi bodies	Nucleolar extrusions.	Yes.
Helix ...	Gatenby ...	Golgi bodies	Probable ..	None.
Patella ...	Ludford .	Golgi bodies	...	Yes.
Pila globosa	Bhattacharya and Lal.	Golgi bodies	Nucleolar extrusions.	Yes.
Ostrea ...	Rai H R. Singh.	Golgi bodies	Absent ...	None.
Calanus .	Hilton, I. F.	None . .	Mitochondria	Yes.
Ophiocephalus.	Narain, D.	Golgi bodies	Mitochondria	Yes.
Discoglossus.	Hibbard, H.	*De novo* in the cytoplasm.	Golgi bodies	...
Rana ..	Narain, D.	Golgi bodies	Mitochondria	Yes.
Tortoises	Bhattacharya	Golgi bodies	Mitochondria	Yes

Genus or animal.	Author	Fatty yolk	Albuminous yolk	Nucleolar extrusions, if present.
Fowl ...	Brambell ..	Possibly under the influence of Golgi bodies	Mitochondria	None.
Birds (Pigeon).	Das, R. S.	Golgi bodies	Mitochondria	None.
Lepus .	Del Rio Hortega	Mitochondria and Cytoplasm.	.	...
Lemur ..	Rao, S.	Nucleolar omissions.	Mitochondria and Golgi bodies	Yes.

SUMMARY

(1) The Golgi apparatus in Scylla Serrata is revealed best by Da-Fano's Cobalt nitrate method and Ludford's technique.

(2) The Golgi apparatus consists of discrete crescent-shaped or spherical bodies as revealed in fixed preparations as well as by Intra-vitam examinations. The spherical Golgi body may be differentiated into a chromophilic rim and a chromophobic centre while the dictyosome appears as an osmiophilic crescent attached to an archoplasmic area.

(3) In the oogonial stages the Golgi elements appear in the form of a few black granules lying in the clear and homogeneous cytoplasm adjacent to the nucleus.

(4) In the early oocytes the Golgi elements form a juxtanuclear complex mass—the "Yolk-nucleus of Balbiani" which has been regarded as the focus of growth and dispersal of Golgi bodies.

(5) Gradually, the Golgi elements begin to get detached from the compact mass and disperse in the cytoplasm till in a full-grown oocyte these bodies are seen scattered throughout the cytoplasm.

(6) During the formation of Yolk-nucleus some Golgi elements swell up to form fatty yolk bodies by deposition of fat inside the chromophobic or archoplasmic area

(7) Fatty yolk bodies appear as solid black bodies in Osmic preparations and are readily dissolved when treated with turpentine leaving clear vacuoles with an osmiophilic rim or a crescent.

(8) Mitochondria are revealed best by Regaud, Regaud-Tupa and Champy-Nassonov techniques.

(9) Like the Golgi elements they also occur in the "Yolk-nucleus" area, whence they migrate round the nucleus till gradually they disperse throughout the cytoplasm. They are always granular and dusty and have never been noticed to swell up No filamenter forms have been observed

(10) Nucleolar phenomenon is best exhibited in Bouin preparations stained with Mann's methyl-blue-eosin

(11) From the very beginning the nucleol have a tendency to plaster themselves to the nuclear membrane leaving behind

a prominent basophil nucleolus The former pass out into the cytoplasm as such or fragment into nucleolar extrusions

(12) The basophil nucleolus gives out basophil bodies which pass out from the nucleus into the cytoplasm. During this period they get transformed into oxyphil nucleolar extrusions which may indirectly influence the synthesis of proteid yolk.

(13) Occasionally, the whole of the nucleolus is seen to come out of the nuclear membrane into the ooplasm without apparently rupturing the nuclear wall The significance of this is unknown but probably it buds off nucleolar extrusions in the ooplasm.

(14) The true yolk or proteid yolk is formed "de novo" in the cytoplasm Mitochondria play absolutely no part in vitellogenesis

(15) In the Intravitam examinations, patches of neutral red staining vacuoles have been observed which lie quite distinct and separate from the Golgi bodies These are the patches of Parat's "Vacuome" Their behaviour and functions are yet to be discovered

EXPLANATION OF PLATES

The drawings were made under Leitz Abbe Camera Lucida.

Figs. 1-6. Da-Fano preparations stained in Safranin and Light green. Sections were cut 6 μ in thickness

Fig. 1. The young oogonial stage showing a few discrete Golgi granules.

Figs. 2 and 3. Early oocytes in which the Golgi granules have a tendency to lie on one side of the nucleus.

Fig. 4. An oocyte showing the "Yolk-nucleus of Balbiani" formed by the Golgi bodies.

Fig 5 An oocyte in which the Golgi bodies have begun to detach themselves from the Yolk-nucleus of Balbiani, and scatter in the cytoplasm.

Fig 6. Showing a well advanced oocyte in which the Golgi elements have scattered throughout the cytoplasm in the form of vesicles and crescents Fatty yolk bodies are also visible, formed by the Golgi bodies.

Figs. 7-14 Ludford preparations stained in Champy-Kull.

Fig. 7 represents an early oogonium showing two or three Golgi granules in the clear homogeneous cytoplasm

8-10 represent early oocytes in which the Golgi granules increase in number and lie juxta-nuclear in position.

11 An oocyte showing the formation of the "Yolk-nucleus of Balbiani."

12. An oocyte where the Golgi bodies give rise to some fatty yolk bodies. The yolk-nucleus has begun to disseminate in the cytoplasm.

13. Showing the detached Golgi vesicles and dictyosomes scattered throughout the cytoplasm around the nucleus.

14 Showing a fully developed oocyte in which the Golgi elements have dispersed throughout the cytoplasm. Besides the fatty yolk bodies, some albuminous yolk bodies are also seen, stained cherry red in acid fuchsin

15-23. Champy-Nassonov preparations stained in Champy-Kull. Sections 5 μ in thickness.

15. An advanced oogonium showing two or three dusty granules of mitochondria.

16 and 17 represent early oocytes where the mitochondrial granules increase in number and lie adjacent to the nucleus.

18 An oocyte where the mitochondria occur in the "Yolk-nucleus of Balbiani." Some albuminous yolk bodies are also visible stained in acid fuchsin.

19 and 20 represent oocytes where the mitochondrial granules detach from the yolk nucleus and form a peri-nuclear zone.

21, 22 and 23 represent the well advanced oocytes where the mitochondria increase in number and scatter throughout the cytoplasm. Albuminous yolk bodies are fairly big in size and dispersed in the cytoplasmic area

24-27. Regaud preparations stained in Champy-Kull Sections 5 μ in thickness.

24 An early oocyte showing some mitochondrial granules lying adjacent to the nucleus.

25 Showing the formation of the "Yolk-nucleus of Balbiani."

26 and 27 Showing the dispersal of the mitochondria in the cytoplasm as in Champy-Nassonov preparations.

28 31 Bouin preparations stained in Mann's methyl-blue eosin Sections 5 μ in thickness

28 Showing an oocyte in which there are many nucleoli within the nucleus, and one of them being prominent

29 Showing an oocyte where the nucleoli plaster themselves round the nuclear membrane, leaving a prominent basophil nucleolus stained deep red in Mann's methyl-blue-eosin

30. Showing some oxyphilic nucleolar extrusions in the cytoplasm coming through the nuclear membrane Oxyphil bodies are stained lighter in the stain.

31. Showing a well advanced oocyte in which the cytoplasm is fully packed with the nucleolar extrusions.

32-34 represent the Fatty yolk formation Da-Fano preparations stained in Safranin-Light green.

32 Showing the vacuolar fatty yolk spheres each with an associated Golgi crescent or vesicle, in the region of the yolk-nucleus.

33 and 34 Showing the oocytes where the intermediate stages between the Golgi elements and the fatty yolk bodies are seen (swollen up Golgi bodies).

35 shows a Champy-Nassonov preparation stained in Champy-Kull. Representing the albuminous yolk bodies stained cherry red in acid fuchsin

36 represents the centrifuged material fixed in Champy-Nassonov followed by Champy-Kull. Three separate zones of mitochondria, albuminous yolk, and Golgi bodies with associated fatty yolk bodies are visible individually

37 and 38 represent oocytes in "Bouin" stained with Mann's methyl-blue-eosin, showing the occasional shifting of the nucleolus into the cytoplasm apparently without rupturing the nuclear wall. In the cytoplasm it presents an oxyphilic consistency.

39, 40, 41 represent oocytes as seen in the Intra-vitam examination with Neutral red. These represent the structure and dispersal of the 'Vacuome' patches, formed of congeries of small vacuoles stained red in neutral red.

42 and 43 represent oocytes studied afresh in 2 per cent osmic acid. These show the fatty yolk bodies and the Golgi crescents and vesicles with an osmiophilic rim and a clear chromophore area

LETTERING

Gb	.	Golgi bodies.
Gb_1.	...	Swollen up Golgi bodies.
F.	...	Fat.
F.y.	.	Fatty yolk.
M.	..	Mitochondria
A y.	..	Albuminous yolk.
N.	...	Nucleus.
Nu.		Nucleolus.
N E.	..	Nucleolar extrusions.
Y Nuc.	..	Yolk-nucleus of Balbiani.
B Nu	. .	Basophil nucleolus
O. Nu.	.	Oxyphil nucleolus.
A Nu.	..	Amphophil nucleolus
Vc.	..	Vacuome

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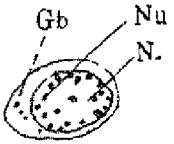

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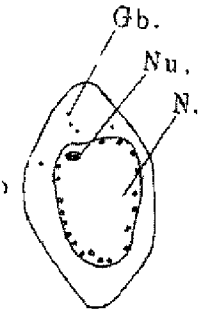

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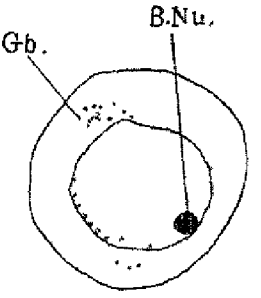
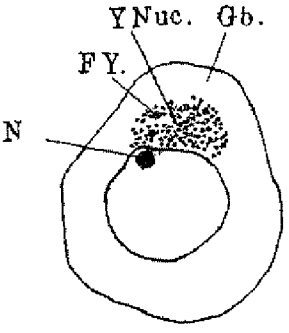


Fig.3



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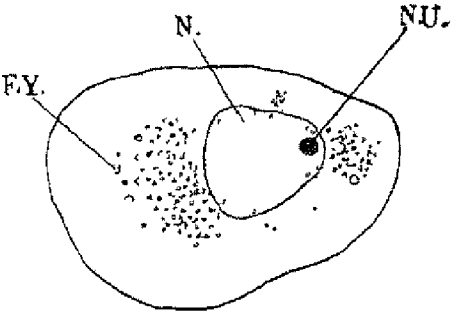

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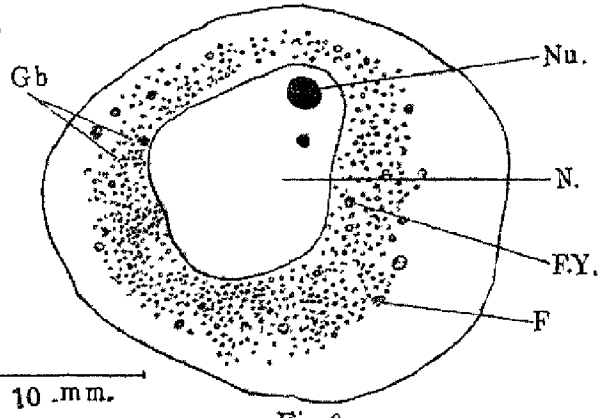

Fig 6
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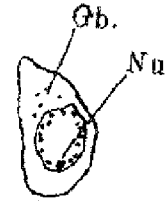
Fig.7

Fig.8.



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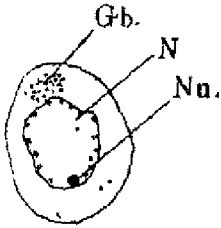

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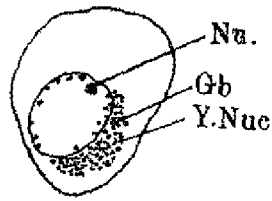

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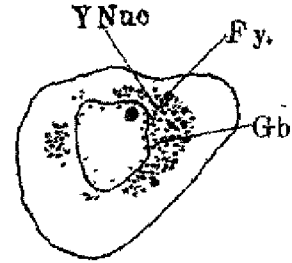

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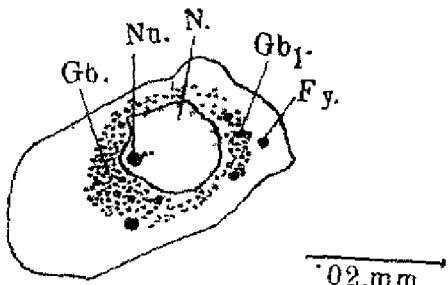

Fig 13.

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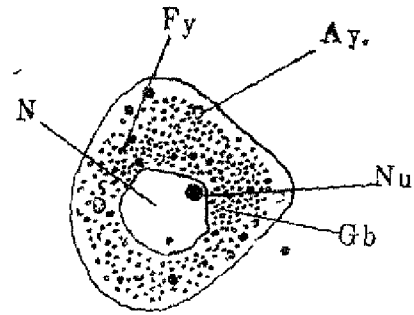

Fig.14.

Ludford-Nassonov-

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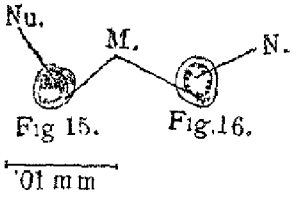

Fig 15. Fig.16.

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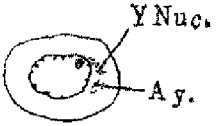
Fig. 17.



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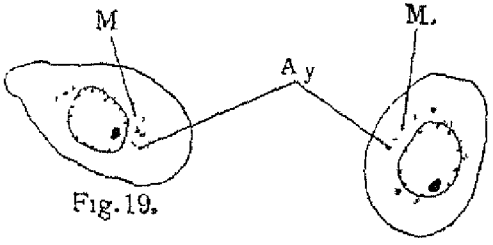

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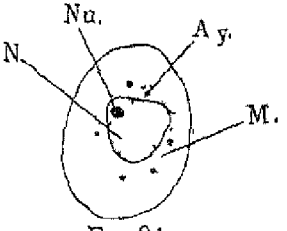

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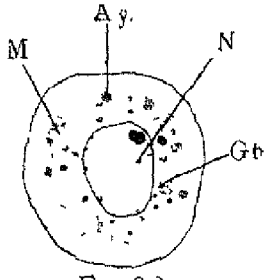

Fig. 22

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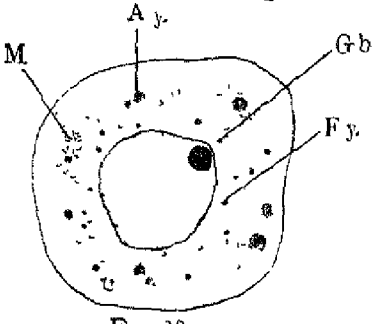
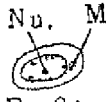


Fig 23.

Champy-Kull.



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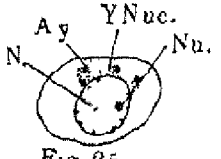

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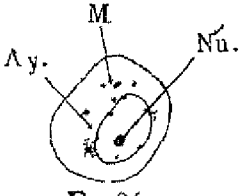

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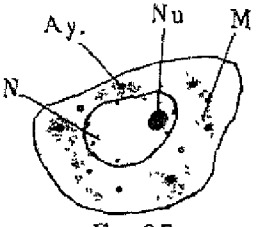

Fig 27

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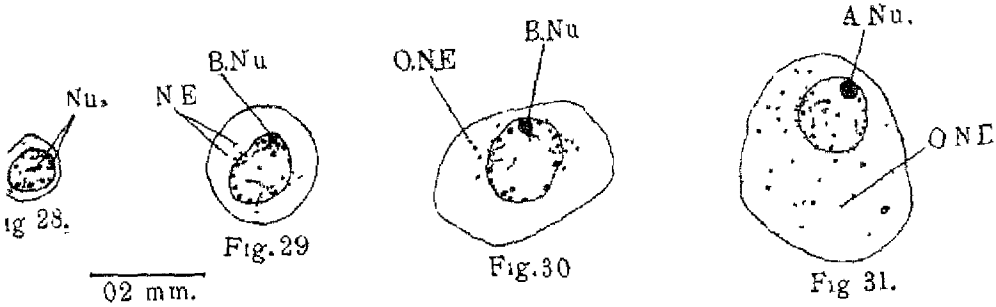
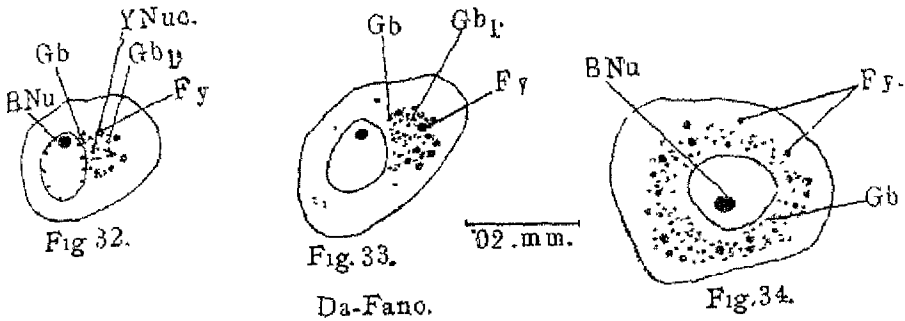
Plate No. 4



Bouin-Mann's Methyl-Blue Eosin



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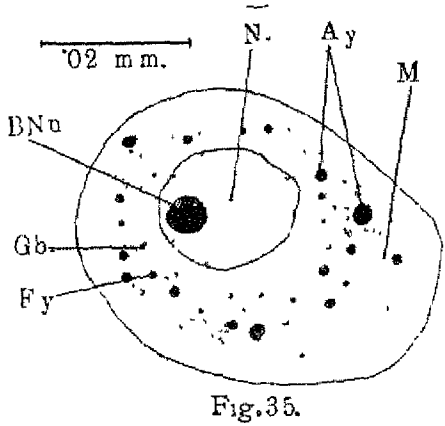


Champy-Nassonov-Champy-Kull.

Plate No 5

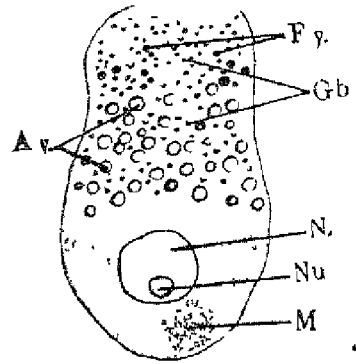


Fig 36.

Centrifuge-Champy-Nassonov
Champy Kull

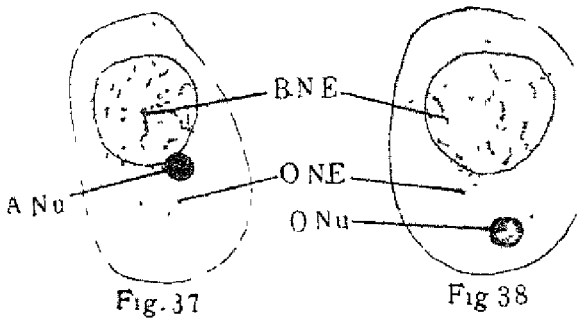
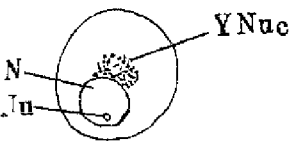


Fig. 37 Fig 38

02 mm Bouin-Mann's Methyl-Blue Eosin



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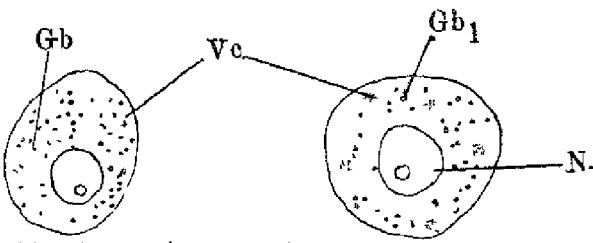

Fig 40 02.mm. Fig. 41.

Intra Vitam-Neutral Red

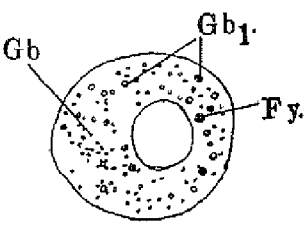

Fig. 42.

02.mm.

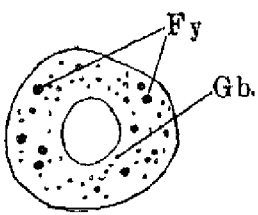

Fig. 43.

Intra Vitam 2% Osmic Acid.

SECTION II

CHEMISTRY

IODINE VALUE OF SATURATED FATTY ACIDS AND THEIR SALTS

BY

HAR KUMAR PRASAD VARMA, M Sc.,

Research Scholar, Chemistry Department.

In 1898 Wijs[1] proposed a method for the determination of Iodine Value in which iodine trichloride was made to be absorbed by oils in presence of glacial acetic acid. Hübl, Winkler and others[2] have proposed alternative methods for the determination of iodine numbers and since then, various investigators have determined the iodine number for almost all vegetable and animal fats, and unsaturated fatty acids Up till now it had been supposed that unsaturation is an essential condition for the absorption of iodine and this was the reason why only the unsaturated fats and oils have been investigated.

In a previous communication from this laboratory, Palit and Dhar[3] studied the oxidation of the salts of saturated fatty acids by passing a current of air through their solutions in presence of various inductors in tropical sunlight. They found that the amount of the fat thus oxidised can easily be estimated by determining the amount of absorption of iodine trichloride by fat before and after the experiments. From these results we are led to think that

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[1] Wijs . Ber, *31*, 750 (1898) ; Ztschr. Nahr. U Genus, *4*, 913 (1901).

[2] Rowland Williams: J. Soc. Chem. Ind., *19*, 300 (1900), Tolman and Munson J. Amer. Chem. Soc., *25*, 244 (1903); Aschman: Chem. Ztg., *22*, 59, 71 (1898) ; Margosches, Baru and Wolf: Z. Anal Chem., *62*, 178 (1923), Weiser and Donath Zeitsch. Nahr. Genussm, *28*, 65 (1914).

[3] Palit and Dhar: J. Phys. Chem., *34*, 711 (1930).

saturated fatty acids do also possess a specific iodine number, howsoever small it may be, in comparison to that of the unsaturated compounds.

In the present paper, I have thoroughly investigated this problem and have determined the iodine value of the following substances :—

Propionic Acid.
Sodium Propionate.
Butyric Acid.
Sodium Butyrate.
Stearic Acid
Potassium Stearate.
Palmitic Acid.

EXPERIMENTAL

In the determination of the iodine value the procedure recommended by Wijs was followed. The following reagents were prepared :—

1. Iodine Solution—This was prepared by dissolving thirteen grams of iodine in one litre of glacial acetic acid. Pure glacial acetic acid, which did not give a green colour on heating with potassium dichromate and sulphuric acid after prolonged standing (for about six to eight hours), was used. 10 c.c. of this solution were titrated with a standard solution of sodium thiosulphate. Chlorine, purified and dried by washing through concentrated sulphuric acid was led into the iodine solution, till it changed colour and its iodine content was doubled. The solution was kept for 24 hours before being used (Lewkowitch).

2. Standard sodium thiosulphate solution—It was prepared by dissolving a carefully weighed amount of the

pure salt in distilled water and making it up to a known volume. A fairly dilute solution of about 0·025N strength was employed.

3. A 10·0 per cent potassium iodide solution was used.

4. A fresh starch solution of about 1·0 per cent strength was prepared for use as an indicator, by pouring an emulsion of starch in cold water in nearly boiling water contained in a beaker, and stirring it.

A carefully weighed quantity of the pure fatty acid or one of its salts was dissolved in some pure glacial acetic acid, and the solution was then made up to 100 c.c by adding the iodine solution as prepared above. Immediately after preparation, 10 c c. of this solution was taken in a glass-stoppered Jena bottle, 10 c.c. of a 10·0 per cent potassium iodide solution and 25 c.c. water added, and the whole titrated with a standard solution of potassium thiosulphate. This gave the blank reading. 10 c.c. of the solution of the fat were taken in each of a number of glass-stoppered Jena bottles and kept in an electrically-driven rocking machine for a number of hours in order to keep the solution in a constant state of agitation throughout. The bottles were covered with a thick dark-coloured paper to protect them from diffused light. They were taken out as required, 10 c.c of a 10 per cent potassium iodide solution and 25 c c. water added, and the whole titrated with a standard sodium thiosulphate solution. From this data, the iodine value was calculated in the usual manner.

TABLE I

Absorption of Iodine by Propionic Acid

8·1228 gms. of propionic acid was dissolved in glacial acetic acid, and the solution was then made up to 100 c.c. with the iodine solution as prepared above.

Concentration of the sodium thiosulphate solution used = N/38 29

10 c.c. of the above solution required 21·0 c.c sodium thiosulphate (Blank)

10 c c. of the above solution were titrated with hypo after shaking mechanically for :—

Hours	N/38 29 hypo required	Iodine Value
15	20 4 c.c.	0 25
24	20·3 c.c	0·29
41	17 7 c.c	1 35
47	17 25 c.c	1 53

Before each titration, 10 c c. of a 10 per cent potassium iodide solution and 25 c.c. water were added to each bottle. The starch solution was added near the end of the titration when the colour of the solution became very pale.

TABLE II

Absorption of Iodine by Sodium Propionate

1·5518 gms. of sodium propionate was dissolved in glacial acetic acid, and the solution was made up to 100 c c. by adding iodine solution.

Concentration of the hypo-solution used = N/38·29.

10 c.c. of the above solution were taken in a glass-stoppered bottle, 10 c.c. of a 10 per cent KI solution and 25 c.c. water were added to it. It required 12·3 c.c. of N/38·29 $Na_2S_2O_3$ (Blank).

10 c.c. of the fat solution were taken in each of the two glass-stoppered bottles and were s aken mechanically

for a number of hours. After that 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ required	Iodine Value
6	7·8 c.c	0 96
22	7·2 c.c.	1·1

TABLE III

Absorption of Iodine by Butyric Acid

1·6369 gms. of butyric acid were dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution.

Concentration of $Na_2S_2O_3$ solution used = N/38·29.

10 c.c. of the solution of the fat were taken in a glass-stoppered bottle, 10 c.c. of a 10 per cent KI solution and 25 c.c. water were added to it It required 27·5 c.c. of N/38·29 $Na_2S_2O_3$ (Blank).

10 c.c. of the fat solution were taken in each of the three glass-stoppered Jena bottles, and were shaken mechanically for a number of hours. After that, 10 c.c. of 10 per cent KI and 25 c.c. water were added to each bottle, which were then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ required	Iodine Value
24	27·25 c c.	0·43
47	27·10 c.c.	0·69
72	26·90 c.c.	1·13

TABLE IV

Absorption of Iodine by Sodium Butyrate

1·5767 gms. of sodium butyrate were dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution

Strength of sodium thiosulphate solution N/38·29

10 c.c. of the above solution were taken in a glass-stoppered bottle, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to it. It required 11·4 c.c. of N/38·29 $Na_2S_2O_3$ (Blank).

10 c.c. of the solution of the fat were taken in each of the four glass-stoppered bottles, which were shaken mechanically for a number of hours. After that, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ used	Iodine Value
6	8·3 c.c.	0·65
23	8·2 c.c.	0·67
29	8·2 c.c	0·67
45	8·0 c.c.	0·72

TABLE V

Absorption of Iodine by Stearic Acid

1·2357 gms. of stearic acid was dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution.

Concentration of $Na_2S_2O_3$ solution used = N/38·29. 10 c.c. of the fat solution were taken in a glass-stoppered bottle, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to it. It required 27·3 c.c. of N/38·29 $Na_2S_2O_3$ (Blank).

10 c.c. of the solution of the fat were taken in each of the three glass-stoppered bottles, which were shaken mechanically for a number of hours. After that, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ used	Iodine Value
16	26·9 c.c.	1·4
25	26·8 c.c.	1·7
39	26·6 c.c.	2·4

TABLE VI

Absorption of Iodine by Palmitic Acid

1·0983 gms. of palmitic acid was dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution.

Strength of $Na_2S_2O_3$ solution = N/38·29

10 c.c. of the fat solution was taken in a glass-stoppered bottle, 10 c.c of 10 per cent KI solution and 25 c.c. water were added to it. It required 42·9 c.c. of N/38·29$Na_2S_2O_3$ (Blank).

10 c.c. of the solution of the fat were taken in each of the four glass-stoppered bottles, which were shaken mechanically for a number of hours. After that, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ used	Iodine Value
15	41·9 c.c.	0·30
24	41·7 c.c.	0·36
38	41·3 c.c	0 48
50	40·8 c c.	0·63

TABLE VII

Absorption of Iodine by Potassium Stearate

0·5642 gms. of potassium stearate was dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution.

Concentration of $Na_2S_2O_3$ solution used = N/44·9

10 c.c. of the fat solution was taken in a glass-stoppered bottle, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to it. It required 27·2 c.c. of N/44·9$Na_2S_2O_3$ (Blank)

10 c.c. of the solution of the fat were taken in each of the three glass-stoppered bottles, which were shaken mechanically for a number of hours. After that 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/44·9$Na_2S_2O_3$ used	Iodine Value
31	25·5 c.c.	0·85
49	24·7 c.c.	1·25
73	24·1 c.c.	1·55

DISCUSSION

From the results recorded in the previous tables, it would be seen that sufficient quantity of iodine is absorbed by such saturated compounds as butyric acid, propionic acid, stearic acid, and palmitic acids and also by their sodium and potassium salts. The experiments have been repeated many times and the possibility of the experimental error has been avoided as far as possible. It can be said with confidence that saturated acids also possess an iodine number similar to that in the case of unsaturated acids. It is also clear from my observations that the iodine value of these substances continuously increases with time. It is only after the lapse of a considerable time, that the iodine value, in the case of saturated compounds, attains a maximum. In the case of unsaturated compound, it has been generally observed that the maximum condition is reached within a few hours.

Margosches, Friedmann, Tschörner[4] observed something like the iodine super-value in the case of olive, castor and linseed oils and oleic, ricinoleic, and linoleic acids in aqueous alcoholic solutions. It shows that unsaturated acids are,

[4] Margosches, Friedmann and Tschörner, Ber, *58* (B), 794 (1925).

in the first instance, converted into saturated iodine compounds which are capable of further oxidation. I am of opinion that the iodine number for the saturated acids can also be explained in a similar way. The mechanism proposed by the above authors for the super-iodine value is as follows :—

(1) $I_2+H_2O=HI+HIO$.

(2) $R'CH:CH.CH_2R''+HIO=R'CHI.CH(OH).CH_2R''$.

(3) $R'CHI.CH(OH).CH_2R''+HIO=R'CHI.CH(OH).CH(OH)R''+HI$.

In the first step iodine is actually added to the unsaturated molecule, while in the second step the iodine acts as an oxidising agent. Similarly, the iodine absorption of saturated fatty acids can be explained in the following way :—

(1) $H_2O+I_2=HI+HIO$.

(2) $RCH_2CH_2COOH+HIO=RCH_2CH(OH).COOH+HI$.

The above authors also observed that for the super-iodine value the substances must remain in contact for twenty-four hours or a longer period. The iodine numbers which I have obtained for saturated compounds do not exceed 2·5 in value, while in the case of unsaturated substances they are often as high as 200. This very fact shows that the iodine number of saturated compounds is not primary, but secondary.

The iodine value has been, up-till now, associated with unsaturation and it has been regarded as a reliable method for the estimation of unsaturation. It may be stated, however, that the iodine value, thus determined, gives the combined values due to unsaturation and the secondary oxidation of saturated compounds.

The results recorded in the previous tables also show that there exists no relationship between the iodine value

and the number of carbon atoms of the saturated fatty compound Acetic acid does not appear to absorb iodine to any marked extent even when kept in contact for 45 hours as is seen from the following results :—

TABLE VIII

Four bottles were taken each containing 5 c.c. iodine solution as prepared above. They were titrated with N/38·29 hypo after a number of hours.

No of hours	N/38 29 hypo used.
Immediately	22 55 c.c.
After 20 hrs	22·6 c.c.
After 26 hrs.	22·6 c c.
After 45 hrs	22·6 c c.

However, as soon as the number of carbon atoms increases, the power of absorbing iodine is at once exhibited. It is strange that propionic and stearic acids possess a remarkably higher value than the butyric and palmitic acids. In the case of propionic and butyric acids, the sodium salts possess higher value than the corresponding free acids. From this it appears that sodium salts are more liable to oxidation.

SUMMARY

1. The iodine values for the following saturated fatty acids have been determined :—propionic, butyric, palmitic and stearic

2. It has been shown that the saturated fatty acids also possess an iodine value, which, however, continuously increases with time.

3 The iodine value of the sodium salts of the above acids has also been determined, and it has been shown that the salts generally possess a higher value than the corresponding acids.

4 There appears to be no relationship between the iodine value and the constitution of the compound.

5. A mechanism for the absorption of iodine has been suggested and it is believed that the iodine value is not due to the addition of iodine to the structure of the molecule, but to the oxidation of the methylene group.

FORMATION OF INORGANIC JELLIES

BY

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The number of inorganic jellies so far known is very limited. The organic jellies as of starch, gelatin, soap, agar, pectin, numerous dyestuffs and celluloses are now of classical interest. The phenomenon of spontaneous gelation has also been observed in nature in the process of clotting of blood and formation of curds. As chemists, we have always taken interest in the imitation of nature and it was not unnatural to think that the process of jelly formation, so familiar with the natural-occurring organic substances, could be repeated with success in the case of inorganic salts.

In this communication, we will give in brief the preparation of numerous inorganic jellies so far discovered. For convenience, the jellies may be divided into the following groups :

(1) The jellies of oxy-acids.
(2) The jellies of basic hydroxides.
(3) The jellies of the salts of polybasic acids—
  (*a*) Arsenate jellies.
  (*b*) Phosphate jellies.
  (*c*) Molybdate jellies.
  (*d*) Tungstate jellies.
  (*e*) Borate jellies.
(4) The jellies of sulphides.
(5) Von Weimarn jellies.

The Jellies of Oxy-Acids

Silicic acid, vanadic acid, titanic acid and molybdi acid are the familiar instances of oxy-acids which are know to give jellies

Silicic Acid.—About a century ago, Berzelius[1] prepared silicic acid jelly. However, it was Holmes[2] who for the first time undertook the systematic study of this substance. Silicic acid jelly is prepared by the addition of various acids to the solution of sodium silicate of different concentrations. It has been observed that the gel is rapidly prepared by using slightly less acid than is required to neutralise sodium silicate. Waterglass of 1·15 D and equal volume of 6 N hydrochloric acid gives an excellent gel; however, the concentrations can be varied to a great extent. Holmes[3] further studied the various properties of this jelly including the influence of temperature, and also the syneresis.

Bhatnagar and Mathur[4] observed that silicic acid jelly can also be prepared by the use of the regulated concentrations of sodium silicate and ammonium acetate. Mata Prasad and Hattiangadi[5] extended this work and they have shown that the gel can be obtained by the use of any ammonium salt like chloride, nitrate or sulphate of ammonia instead of ammonium sulphate. They used the concentrations of about 3-5 per cent SiO_2 from Na_2O, 2·33 SiO_2 and 0·5-0·7 N ammonium salts.

Vanadic Acid.—Colloidal vanadic acid was prepared by Prandtl and Hess,[6] and Rideal[7] and its coagulation and optical birefractive properties were investigated by Freundlich and co-workers,[8] Reinders[9] and Kruyt[10] but its property to form a gel was probably for the first time noted

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[1] Ann. Chim. Phys. (3) *38*, 312 (1833).
[2] J. Phys. Chem., *22*, 510 (1918)
[3] J Amer. Chem. Soc , *41*, 1329 (1919).
[4] Kolloid-Z., *30*, 368 (1922).
[5] J. Indian Chem. Soc., *6*, 653, 893 (1929); *7*, 341 (1930).
[6] Z. Anorg. Chem., *82*, 103 (1913).
[7] Chem. Zentr., *1*, 1738 (1914).
[8] Koll. Chem. Beihfte, *7*, 172 (1915).
[9] Proc K. Akad Wetensch Amsterdam, *19* 189 (1916)
[10] Ibid. *18* 1625 (1916)

by Wiegner, Magasanik and Gessner [1] They also observed its close analogy with fibrin sols Vanadic acid sol is prepared by carefully mixing in a mortar some ammonium vanadate and a few drops of concentrated hydrochloric acid, and then washing the red precipitate many times by decantation The acid when sufficiently purified, on further washing passes to the colloidal condition. The sol thus prepared is now allowed to dialyse for a few days. The sol is negatively charged and can be set to jellies by the use of different concentrations of potassium or barium chlorides. The time of the setting of the jelly depends upon the concentration of coagulating electrolytes. Quite transparent jellies are obtained, some of which are very stable, while others undergo syneresis

Titanic Acid.—Graham [2] obtained a sol of hydrous titanic oxide by the dialysis of 1 per cent solution of the oxide in dilute hydrochloric acid With more concentrated solutions, it was observed that the jellies are formed on the dialyser during dialysis. The water in an aged jelly can be replaced by alcohol, ether, benzene, glycerine or concentrated sulphuric acid.

Rose [3] formed the titania jelly by treating a fusion of titania and sodium carbonate with hydrochloric acid, filtering the solution and allowing it to stand whereupon the hydroxide precipitated out as a gel. Knop [4] obtained a jelly as follows .—A strong hydrochloric acid solution of magnetic oxide of iron ($FeTiO_3$) was treated with tartaric acid and neutralised with ammonia. The iron remained in the solution and titania came down as a white precipitate On filtering and attempting to wash the oxide, it swelled up much in the same manner as gelatin forming a

[1] Kolloid-Z., *30*, 145 (1922)
[2] Phil. Trans *151* 213 (1861)
[3] Gilbert's Ann *73* 6 (1823
[4] Liebig's Ann Chem *123* 301 186

colourless transparent jelly On warming however the jelly was transformed to a gelatinous precipitate.

Klosky and Marzano[1] prepared firm transparent jellies by neutralising slowly an acid solution of titanium dioxide with sodium, potassium or ammonium carbonates Recently, Bhatia and Ghosh[2] obtained a sol of titanic acid by dialysing a solution of titanium tetrachloride in water (*Cf.* Majumdar, J. Indian Chem. Soc, *6*, 357 (1929). They observed that this sol sets to a jelly by the addition of electrolytes. The sol prepared by them, however, appears to be positively charged.

Molybdic Acid.—Not much work appears to have been done on molybdic acid jellies. It has been observed that on addition of suitable concentrations of hydrochloric acid to a strong solution of ammonium molybdate, the molybdic acid is precipitated in the form of a solid opaque jelly.

The Jellies of Basic Hydroxides

The most common of the inorganic hydroxide jellies are those of iron, chromium, aluminium, tin, zirconium, copper, mercury, manganese, scandium, erbium, cerium and non-aqueous gel of nickel.

Ferric Hydroxide Jelly.—Grimaux[3] added an alcoholic solution of ferric ethylate to an excess of water, which on hydrolysis yielded colloidal ferric oxide. The sol coagulated spontaneously on standing for some time at the room temperature and more rapidly on heating or adding electrolytes, like potassium or barium chlorides or sulphuric acid, in some cases forming a transparent jelly, provided the sol is not agitated during the coagulation. Even dilute sols gave firm jellies. Contraction took place in the jellies, however, in the cold and this too, very rapidly at higher temperatures.

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[1] J. Phys. Chem. *29*. 1125 (1925)
[2] J Ind an Chem Soc *7* 687 1930
[3] Compt Rend *98* 105 1434 1884

The ferric hydroxide sol prepared by the Graham's method does not give jellies, but if the concentration be sufficiently high, a jelly may be formed. Schalek and Szegvary[1] added electrolytes in amounts below their precipitation values to the colloidal solutions containing 6 to 10 per cent ferric oxide and allowed the sols to stand quietly. After a time, the mixture set to a jelly which was almost as transparent as the original sol. This jelly slowly developed opalescence. It also exhibited the phenomenon of thixotropy, *i.e.*, the gel on shaking was transformed to sol which on standing re-formed the gel.

Grimaux[2] obtained a firm jelly by dialysis of a negative sol prepared by peptisation of hydrous oxide with alkali in the presence of glycerine. If ammonia were used instead of caustic alkali, and the sol exposed to air, the slow loss of the peptising agent by evaporation, also resulted in the precipitation of a jelly. Fischer[3] prepared a firm jelly by the prolonged dialysis of a sol containing but one per cent of iron Browne obtained a jelly simply by allowing a part of the water to evaporate slowly from a concentrated Graham sol of high purity.

Dhar and Chakravarti[4] observed that various metallic hydroxide jellies can be prepared by adding sodium acetate to a metallic nitrate or chloride, and allowing the hydroxide to coagulate in the presence of ammonium sulphate, and also regulating the hydrogen ion concentration by the addition of suitable amounts of ammonia.

In a recent communication, Prakash and Dhar[5] have investigated the formation of this jelly by the above method in details. The jelly has been prepared by adding varying amounts of 3·54 N sodium acetate to M/2 ferric chloride

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[1] Kolloid-Z., *32*, 318; *33*, 326 (1923).
[2] Compt. Rend., *98*, 1485 (1884).
[3] Biochem Z., *27*, 223 (1910).
[4] Z Anorg Chem *168* 209 1927)
[5] J Indian Chem. Soc *7* 591 (1930)

solution in presence of small amounts of 2M ammonium sulphate and then adding a trace of ammonia. Some of the suitable concentrations for the formation of this jelly are given below.

TABLE I

Total Volume—5 c.c.

M/2 $FeCl_3$	3·54N Sodium acetate	2M Ammonium sulphate	5·81N Ammonia	Observation
c.c.	c.c	c.c.	c.c	
2·0	0·9	0·5	0·1	Clear solution no jelly.
2·0	1·0	0·5	0·1	Turbid solution, no jelly.
2·0	1·2	0·5	0·1	Loose jelly in 20 hrs.
2·0	1·3	0·5	0·1	Firm opaque jelly in 20 hrs.
2·0	1·5	0·5	0·1	Opaque solution, no jelly.
2·0	1·2	0·3	0·1	Firm opaque jelly in 22 hrs.
2·0	1·2	0·6	0·3	Firm opaque jelly in 22 hrs.
2·0	1·2	0·8	0·1	Firm opaque jelly in 10 hrs.
1·33	0·8	0·2	0·1	Opaque loose jelly in 22 hrs.
1·0	1·0	0·2	0·1	No jelly, precipitate.
1·0	1·2	0·5	0·1	No jelly, precipitate.

It will be seen from these results that for a given amount of ferric chloride, there is a corresponding minimum amount of sodium acetate which must be added before expecting a jelly. A little quantity above this minimum is always favourable, but the addition of larger amounts of sodium acetate will not form the jelly. As regards the addition of ammonium sulphate, a small quantity is always sufficient, the addition of it beyond a limit gives loose jellies or gelatinous precipitates. The addition of a trace of ammonia is sufficient to give a jelly, if the concentrations of the other constituents are suitable; in some cases, however, it may not be essential at all. The jellies thus obtained by this method are opaque and stable and do not

undergo any marked syneresis, and some of them can be preserved for months.

Chromic Hydroxide Jelly.—Reinitzer[1] observed that a solution of chromic salt boiled with sodium acetate and rendered alkaline with caustic alkalies or ammonia sets to a jelly. Bunce and Finch[2] found that a very satisfactory jelly can be obtained by dissolving one part of chromic chloride or sulphate in 20 c.c of water and adding approximately one gram sodium acetate per 20 c.c solution, and boiling the mixture for 1 minute and then allowing it to cool. The solution is now violet in colour Concentrated solution of caustic potash is now added until the solution is distinctly alkaline. The solution now turns green and sets within 20 minutes. If ammonia is added instead of caustic soda or potash, the solution becomes distinctly purple and sets to a jelly in some 12 hours. They also observed that a jelly is obtained by adding caustic soda to chromic acetate or chrome alum solution. Nagel[3] obtained the chromic hydroxide jelly by regulating the concentrations of chromic sulphate or chrome alum and caustic potash Good jellies were obtained by mixing 50 c.c. of $Cr_2(SO_4)_3$ solution containing 34 gms. of the salt per litre and 10-11 c.c. KOH (1 : 1). Weiser[4] prepared a jelly by adding an excess of caustic alkali to chromic chloride solution, and allowing it to precipitate in the presence of ammonium or potassium sulphate. A green jelly is thus slowly formed. It appears that in the presence of an excess of alkali, the precipitated hydroxide gets peptised and a negatively charged sol is formed, which in the presence of further electrolytes undergoes slow coagulation, and finally a jelly is obtained.

In a recent publication, Prakash and Dhar[5] have investigated in details the conditions of the formation of

[1] Monatsh, *3*, 249 (1883) ; Chem News, *48*, 114 (1883)
[2] J Phys Chem , *17*, 769 (1913)
[3] Ibid *19* 331 (1914)
[4] Colloid Symp Monograph Wisconsin *1* 46 1923
[5] Loc c t

this jelly We have observed that the jel y is best prepared by adding a sufficient amount of 3·54 N sodium acetate to M/2 solution of ferric chloride in presence of small amounts of 2M ammonium sulphate. The mixture is allowed to stand for about an hour and then some ammonia is added to it. If the mixture is not allowed to stand for sufficient time before the addition of ammonia, it will cause an immediate precipitation and no jelly would be obtained

The clear mixture thus obtained develops opalescence and if the concentrations of the constituents are suitable, a jelly would be formed. The time of setting of the jelly would depend upon the relative concentrations of the reactants, as is shown in the following table .

TABLE II

Total Volume—5 c.c.

M/2 $CrCl_3$	3·54N sodium acetate	2M Ammonium sulphate	5·81N Ammonia	Observation
c c	c c.	c.c.	c.c.	
2·0	0·7	0·5	0 7	No jelly.
2 0	1·0	0·5	0 7	Translucent jelly in $2\frac{3}{4}$ hrs.
2 0	1·3	0·5	0 7	Translucent jelly in $1\frac{1}{2}$ hrs.
2·0	1·5	0·5	0 7	Clear solution, no jelly
2 0	1·5	0·5	1·0	Firm opaque jelly in 2 hrs.
2 0	1·3	0·2	0·7	Translucent jelly in 1 hr.
2·0	1·3	0·7	0 7	Opaque jelly in 2 hrs.
2 0	1·3	1·0	0 7	Opaque jelly in $2\frac{3}{4}$ hrs.
2·0	1·0	0·5	0·5	Clear solution, no jelly.
2·0	1·0	0 5	1 0	Translucent jelly in 15 hrs.
2·0	1·0	0·5	1·3	Opaque jelly in $2\frac{1}{2}$ hrs.
1·0	1·0	0 5	0 7	Opaque jelly in 22 hrs
0·5	1·0	0·5	0 7	Loose jelly in 24 hrs.

By studying the influence of the variation of the concentration of these reactants it has been observed that as

in the case of ferric hydroxide, a minimum amount of sodium acetate is necessary for a given amount of chromic chloride for the jelly formation. Similarly, the addition of greater quantities of ammonium sulphate always gives the jellies of weaker texture in a longer time. The regulation of the quantities of ammonia is also an important factor in the formation of this jelly ; and a sufficient quantity of ammonia (which is much greater than was necessary for the preparation of ferric hydroxide jelly) has always to be added before a jelly could be expected.

The jellies obtained by dilute solutions of chromic chloride are translucent, but those with concentrated solutions are opaque. The jellies are very stable and of fine texture, and do not undergo any marked syneresis.

The jelly prepared by Weiser's method (loc. cit.) by the addition of the excess of caustic alkali to chromic chloride solution is green and not so fine in texture as obtained by our method. The jellies which we have described are violet in colour and resemble those of Reinitzer, though we have prepared them at the ordinary room temperature.

Aluminium Hydroxide Jelly.—Not much work appears to have been done on this jelly. A sol formed by peptising sufficient amount of hydrous alumina to form a viscous liquid has been observed to set to a jelly on standing. The jelly breaks up on shaking and cannot be re-converted to the gel form. Schalek and Szegvary[1] prepared a sol by Crum's method which set to a jelly on the addition of a suitable amount of electrolyte just below the precipitation value. On shaking, the sol was re-formed which again set to a jelly on standing, thus exhibiting thixotropy. It has also been observed that a jelly may be formed by peptising hydrous alumina with acetic acid but shaking converts the jelly into a gelatinous precipitate that is not re peptised

[1] Kolloid Z. *33* 326 (1923)

The preparation of this jelly by the usual Prakash and Dhar's method[1] requires more regulation of the concentrations of the reactants than iron or chromium hydroxide jellies. To M/2 solution of aluminium nitrate are added varying concentrations of 3·54 N sodium acetate and 2M ammonium sulphate, and then a little of 5·81 N ammonia is added, drop by drop with constant stirring, and thus a clear colourless solution is obtained which soon develops opalescence on standing, and if the concentrations are favourable, firm translucent or opaque jellies are obtained.

In some cases, the opalescence of these jellies increases with time and finally even translucent jellies become opaque. Some of the concentrations for the preparation of these jellies are given below

TABLE III

Total Volume—5 c.c.

M/2 $Al(NO_3)_3$	3·54N Sodium acetate	2M Ammonium sulphate	5·81N Ammonia	Observation
c. c.	c. c	c. c.	c. c	
2·0	1·0	0·5	0·5	Transparent jelly in 3 days.
2·0	1·0	0·7	0·5	Translucent jelly within 22 hrs.
2·0	1·2	0·7	0·5	Precipitate, no jelly
2·0	1·0	1·0	0·5	Translucent jelly in 22 hrs
2·0	1·0	1·2	0·5	Opaque jelly in 22 hrs.
2·0	1·0	0·5	0·6	Translucent jelly in 27 hrs.
2·0	1·0	0·5	0·7	White precipitate, no jelly.
2·0	1·0	0·7	0·3	Transparent jelly in 3 days.
2·0	1·0	0·7	0·4	Transparent jelly in 26 hrs.
1·0	1·0	0·7	0·5	No jelly.
2 (of 0·75M)	1·1	0·7	0·5	Translucent jelly in 2 days.
2 (of 0·75M)	1·1	0·7	0·6	Precipitate no jelly

Log out

There appears to be a very limited range over which the quantity of sodium acetate could be varied. The addition of large amounts of ammonium sulphate slightly decreases the time of setting, but increases the opacity of the jelly. It has also been observed that greater the concentration of ammonium sulphate, the less would be the amount of ammonia necessary to give a jelly. The addition of ammonia in large quantities, however, gives either opaque or loose jellies or precipitates. Aluminium hydroxide jellies prepared by our method are very stable, quite uniform in texture.

Stannic Hydroxide Jelly—It has been observed[1] that when a colloidal solution of hydrous stannic oxide is evaporated, a transparent jelly is obtained, whilst precipitation with electrolytes is said to give always a gelatinous precipitate, but no jelly. Weiser[2] prepared colloidal stannic oxide by Zsigmondy's method, *i.e.*, by allowing a small amount of stannic chloride-hydrate to stand in a large amount of water for three days, and washing the resulted hydroxide by the aid of centrifuge until it was so free from chlorides that it started to go into the colloidal solution. Several of these washed portions were combined, shaken up with water containing a small amount of ammonia, and allowed to stand until the peptisation was complete. The excess of ammonia was removed by boiling which ages the colloidal oxide. The sol obtained in this way was mixed with different amounts of coagulating electrolytes, and allowed to stand for two days Under suitable conditions, this gave transparent jellies, and sometimes only cloudy jellies could be obtained.

We have prepared stannic hydroxide jellies by the addition of varying concentrations of 3·54 N sodium acetate to M/2 solution of tin tetrachloride (liquid Kahlbaum) in

[1] Zsigmondy Spear Chemistry of Colloids 155 1917
[2] J Phys Chem *26* 681 (1922)

presence of small quantities of ammonium sulphate. The solution soon develops opalescence and finally a jelly is obtained. The addition of ammonia is not necessary to obtain this jelly.

In certain cases, it has been observed that where stannic chloride solution is accompanied with free hydrochloric acid as in the cases of hydrated crystals of stannic chloride, or an old solution of tin tetrachloride, the addition of ammonia is also essential to obtain a jelly. Some of the concentrations to give a jelly are given below :

TABLE IV

Total Volume—5 c.c.

M/2 $SnCl_4$	3·54N Sodium acetate	2M Ammonium sulphate	5·81N Ammonia	Observation
c. c	c. c.	c. c	c c	
2·0	0·7	0·5	0	Clear solution, no jelly.
2·0	0·9	0·5	0	Opaque jelly in one day.
2·0	1·0	0·5	0	Opaque jelly in one day, slight syneresis after two days.
2·0	1·2	0·5	0	Immediately opaque jelly, readily undergoing syneresis.
2·0	1·0	0·6	0	Opaque jelly in 14 minutes, syneresis after 5 hrs
2·0	1·0	0·7	0	Opaque jelly in 10 minutes.
2·0	1·0	0·9	0	Opaque jelly in 2 minutes, syneresis soon starts.
2·0	0·7	0·5	0·1	White opaque jelly in 32 hrs.
2·0	0·7	0·5	0·2	White opaque jelly in 22 hrs
2·0	0·7	0·5	0·3	Loose jelly immediately, soon undergoing syneresis.
1·0	0·5	0·1	0	Opaque jelly in 10 minutes.
1·0	0·4	0·1	0	Firm opaque jelly in one day

It has been observed that there is always a limited range over which the quantity of sodium acetate can be extended to give a jelly. The jellies obtained by the addition of large amounts of either sodium acetate or ammonium sulphate begin to break or synerise at once. The addition of ammonia is also necessary where the concentrations of sodium acetate and ammonium sulphate are insufficient to give jellies.

Stannic hydroxide jellies obtained by the above method are opaque. Some of these are very stable, while others break up on ageing.

Zirconium Hydroxide Jellies.—It was, perhaps, for the first time observed by Rosenheim and Hertzmann,[1] and afterwards by Dhar and collaborators[2] that zirconium hydroxide jellies can be obtained by the dialysis zirconia sols. If 10 per cent solution of zirconium nitrate be allowed to dialyse for about a week, a clear sol is obtained which when coagulated with potassium chloride or sulphate yields jellies or gelatinous precipitates according to the conditions. The jellies are perfectly transparent, and if the electrolyte added is not in too much excess, the jellies may be kept as such for months without undergoing marked syneresis If the coagulating electrolyte is added in excess, the jellies rapidly synerise.

I have further observed that unstable translucent jellies of zirconium hydroxide can be obtained by simply adding sodium acetate to zirconium nitrate solution and allowing the mixture to stand for a few minutes. The addition of sulphate ions is not essential, though favourable for the formation of the jellies as has been shown in the following table :—

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[1] Ber *40* 810 (1907)

[2] J Indian Chem. Soc *5* 309 (1928)

TABLE V

Total Volume 5 c c

M/2 Zirconium Nitrate	3·54N Sodium acetate	N K_2SO_4	Observation
c. c.	c. c.	c. c.	
1·0	0·3	0	Clear solution, no jelly.
1·0	0·5	0	Translucent jelly in 2½ mins
1·0	0·7	0	,, ,, 1½ ,,
2·0	0·4	0	Clear solution, no jelly
2·0	0·5	0	Translucent jelly in 4 mins.
2·0	0·7	0	Opaque jelly in ½ min.
3·0	0·5	0	No jelly, clear solution.
3·0	0·7	0	Translucent jelly in 1 min.
4·0	0·7	0.	No jelly, clear solution
4·0	1·0	0	Opaque jelly in 10 secs.
2·0	0·4	0·1	Translucent jelly in 5 mins
2·0	0·4	0·2	Opaque jelly in ½ min
2·0	0·4	0·3	,, ,, 5 secs

The addition of ammonia is not necessary for the preparation of this jelly The jellies obtained by our method are opaque or translucent at the time of setting, but even translucent jellies become opaque on standing for some time. Some of the zirconium hydroxide jellies are stable, whilst others melt, break or synerise after a time. The jellies formed by coagulating the sol obtained by dialysing the solution of zirconium nitrate are far the more transparent and stable.

Copper Hydroxide Jellies—It appears that copper possesses less tendency towards the development of hydration in comparison with the metals so far considered, and as such, it is difficult to prepare good cupric oxide jellies. Perhaps, it was Foerster[1] who for the first time attempted to prepare a jelly of copper. He observed that a jelly is

[1] Ber *25* 3416 1892

formed by the hydrolysis of a solution of cupric ammonium acetate, $Cu(C_2H_3O_2)_2$, $2NH_3$. Finch[1] also made a similar observation. Weiser[2] has reported that much more stable jellies are obtained by precipitation of a suitable amount of colloidal oxide at a suitable rate, *i.e*, by adding ammonia to cupric acetate in the presence of a small amount of sulphate and allowing the unstable colloidal solution to precipitate spontaneously. The solution obtained is perfectly clear at the outset but precipitation starts after intervals varying from a few seconds to several minutes depending upon the relative amounts of the three components. A firm jelly, according to Weiser, which remained unbroken for weeks is obtained by mixing 5 c.c. of 3N ammonia to 25 c.c. of 0·75N $Cu(C_2H_3O_2)_2$ containing 2 c.c. of N-K_2SO_4.

Manganese dioxide Jellies—The sol of manganese-dioxide is evidently lyophobic, and it is difficult to expect a jelly out of it. However, an unstable jelly (which is more or less a sort of pseudo-jelly) has been successfully obtained by Weitzemann.[3] According to his method, 5 gms. of glucose are dissolved in 20 c c. of warm water. The mixture is now cooled in ice-bath. A few c.c. of 10 per cent caustic soda are added and 100 c.c. of potassium permanganate (60 gms. per 1100 c.c.) are added through a dropping funnel in five minutes while agitating the mixture in an ice-bath. The mixture rapidly becomes viscous and in 5 to 10 minutes, sets into a stiff jelly. In such jellies, the syneresis soon starts, and after a few days, a limpid stable sol is obtained. With quite low concentrations, the jelly forms slowly and does not liquefy, while with relatively high concentrations, the jelly stage is not attained, as the highly viscous liquid is rapidly transformed to a sol. In a communication, Prakash

[1] J. Phys. Chem., *18*, 26 (1914).
[2] Ib d *27* 685 (1923
[3] J Amer Chem. Soc *37* 1082 (1915 *39* 27 1917)

and Dhar[1] have studied the nature of the syneresis of this jelly.

Scandium Oxide Jellies.—This jelly has not attracted much attention, simply Bohm and Niclassen[2] made some observations with it By dialysing a solution of scandium chloride, $ScCl_3$, to which ammonia is added short of precipitation, a hydrous sol results which sets to a jelly when treated with suitable amounts of electrolytes It appears that this jelley also exhibits thixotropic property, for on shaking under favourable conditions, it is transformed to a sol condition, and again reversed to a gel state on standing quietly.

Erbium Oxide Jelly.—Bohm and Niclassen[3] used the method of the preparation of scandium oxide jelly in the preparation of erbium oxide jelly They dialysed a solution of erbium nitrate to which ammonia was added short of precipitation The sol thus obtained sets to a jelly on adding a suitable amount of coagulating electrolytes

Ceric Hydroxide Jelly.—Perhaps, Biltz[4] was the first to obtain this jelly, but Fernau and Pauli[5] were the first to make an important investigation of the various properties of the sol and they also observed that β and γ rays from radium act on it in much the same manner as coagulating electrolytes. The sol on the prolonged exposure gives the jelly.

Ceric hydroxide jellies are best prepared by coagulating the sol obtained by the dialysis of a 10 per cent ceric ammonium nitrate for about 5--7 days. Kruyt and van der Made[6] observed that if the dialysis be carried to sufficient extent, the sol sets itself to a firm jelly. This jelly returns to the sol condition if shaken up with a quantity

[1] J. Indian Chem. Soc., 7, 417 (1930).
[2] Z. anorg. Chem *132*, 6 (1924)
[3] Loc. cit
[4] Ber., *35*, 4435 (1902); Z. anorg Chem., *168*, 96 (1927).
[5] Kolloid-Z - *20* 20 (1917)
[6] Rec Trav Chim 4 *42* 277 1923

of freshly dialysed sol. Nitric acid is undoubtedly a peptising agent in the sol, and thus the sol is more stabilised in its presence. Dhar[1] further studied this jelly. It appears that the temperature at which the dialysis is carried has much influence upon the gelation properties of this substance. The higher the temperature of dialysis, the less is the hydration tendency developed by the particles. I have observed that if ceric ammonium nitrate be dialysed at the temperature of tropical summer, the sol yields jellies only with difficulty. The jellies are more readily formed if the coagulation is affected by iodide ions than with chloride or nitrate.

Ceric hydroxide gives transparent jellies, some of which are very stable and can be preserved as such whilst others undergo marked syneresis in the course of time Certainly, this depends on the purity of the sol and the concentration of the coagulating electrolyte used in the preparation of the jelly.

Desai[2] has observed that the time required for the gel formation in the dialyser decreases considerably and the degree of the hydration of the gel increases with the rise in temperature at which the dialysis is carried out. However, I am of the opinion, that as the temperature increases the degree of hydration must decrease provided the other factors are the same In the experiments of Desai, the apparent increase in hydration is not directly due to the increase in temperature, but to the fact that at higher temperatures, the process of dialysis is quicker and thus the sol is more readily purified, and certainly, the greater the purity of the sol, the less would be the time of gelation.

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[1] Chakravarti Ghosh and Dhar Z anorg Chem *164* 63 (1927)

[2] Kolloid Chem Beih *26* 422 1928

Mercuric Oxide Jelly.—Reynolds[1] was the first to observe that mercuric oxide is capable of giving jellies in non-aqueous medium, and this jelly was further studied by Bunce[2] in details. They observed that by adding mercuric chloride to a normal solution of KOH containing 40 c.c. of acetone, a sol is obtained which sets to a firm jelly on standing, the time required depending upon the concentration of the sol.

The easiest method, according to Bunce, is to dissolve 30 gms. caustic potash and 20 c.c. acetone in 500 c.c. water and to add slowly a saturated solution of mercuric chloride. The mixture is continuously shaken, till the appearing precipitate goes on dissolving, and the first faint permanent precipitate occurs. The mixture is allowed to stand for some hours. After a short time, white opalescence develops, and finally, if the concentrations are favourable, white solid opaque jelly is obtained. In some cases, jellies with permanent supernatent liquid are obtained.

Bunce has further observed that the addition of potassium sulphate or sodium nitrate has no apparent effect on the gelation. Addition of potassium carbonate caused the formation of a viscous milky liquid, while cobalt sulphate or copper nitrate caused the formation of a granular precipitate. With sodium acetate, a jelly-like structure was obtained but not a real jelly.

A slight rise in the temperature causes a mixture to gel more quickly, but heating for 5 minutes or more at temperatures above 63° seems to prevent the formation of jellies. Bunce observed that it is impossible to get a jelly if the mercuric chloride is originally contaminated with a mercurous salt.

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[1] Proc. Roy. Soc. *19*. 431 (1871).

[2] J. Phys. Chem. *18* 269 (1914).

Magnesium Hydroxide Jelly.—Recently, Kroger and Fischer[1] have reported the formation of magnesium hydroxide jelly. It has been prepared by adding water to a 3 per cent solution of magnesium ethoxide in methyl alcohol. Such a gel is not very stable and rapidly undergoes syneresis, the more readily the higher the concentration. The gel may be stabilised by the addition of glycerol, glycol, etc., due to the peptising effect. By using water, glycerol and alcohol in proportions of 5 : 10 : 10, a plastic glass clear gel which is stable for months can be prepared Mixed gels have also been obtained.

Nickel Oxide Jelly —Tower[2] has described the preparation of this jelly by dissolving nickel acetate in glycerol to which an alcoholic solution of caustic potash was added. A green gel was obtained by this method, which on standing undergoes syneresis, but the dialysis of this sol once more formed a gel on removal of KOH.

Another method for the preparation of the jelly consists in mixing equivalent concentrations of nickel tartrate and caustic potash. When the solutions are as concentrated as normal, precipitation takes place slowly, giving a transparent green jelly.

Arsenate Jellies

Some of the arsenate jellies rank amongst the best in the whole of our literature The metals which could successfully give arsenate jellies are manganese, zinc, iron, chromium, thorium, tin and cerium.

Manganese Arsenate Jellies.—Deiss[3] has claimed for the priority in the case of this discovery. The jellies are prepared by mixing manganous chloride and potassium arsenate ($KH_2\ AsO_4$) solutions in right proportions Deiss observed that the jellies thus prepared are very stable and can usually

[1] Kolloid-Z., *47*, 5 (1929).
[2] J Phys Chem *26* 733 (1922)
[3] Kolloid Z *14* 139 1914) *15* 16 1915 Z anorg Chem *116* 228 (1921

be kept for weeks without appreciable change. Sooner or later, however, rose-coloured crystals begin to separate. Klemp and Gyulai[1] observed that by the successive addition of ammonium sulphate, acetic acid and excess of sodium arsenate to solutions of zinc, ferrous, manganous, cobalt, cadmium and calcium salts, the colloidal solutions of the arsenates of these metals are obtained in the form of opalescent jellies. Crystals begin to separate from these jellies when kept for some time. In the absence of acetic acid and ammonium sulphate, these solutions yielded only gelatinous precipitates, but no jellies.

The further work on this jelly is of Kraemer[2] and Weiser.[3] Kraemer obtained this jelly by the addition of manganous sulphate to the solution of potassium arsenate He studied the effect of various anions and cations on its gelation. He observed that the lowering of the temperature favours the jelly formation. The time of gelation of this jelly cannot be much extended beyond 10—15 seconds. A slight warming of the solutions always hastens up the beginning of the gelation. It appears that the rise of temperature is necessary to start up the process of jelly formation.

Zinc Arsenate Jellies.—Manganese and zinc arsenate jellies are indistinguishable. Both are perfectly transparent and stable. Sometimes they undergo slight syneresis. Zinc arsenate jellies were for the first time prepared by Klemp and Gyulai.[4] They have obtained this jelly by the addition of potassium dihydrogen arsenate to a solution of zinc sulphate. According to them, disodium or trisodium arsenates, Na_2HAsO_4 or Na_3AsO_4, if previously neutralised by the addition of hydrochloric or acetic acid also yield

[1] Ibid, *15*, 202 (1914).
[2] Colloid Symp. Mono. Wisconsin, *1*, 62 (1923).
[3] J Phys Chem *28* 26 1924
[4] Kolloid-Z *22* 57 (1918

jellies. Crystals appear to separate out of these jellies after two or three months. Weiser[1] has also made some experiments on it.

Ferric Arsenate Jellies.—Manganese and zinc arsenate jellies were prepared by the metathetical reactions of the two salts. However, the method could not be successfully employed in the case of other jellies. The credit of the preparation of excellent jellies of ferric and chromic arsenates goes to Holmes and his co-workers However, Grimaux[2] was the first to obtain this jelly. Holmes and Arnold[3] observed that precipitated ferric arsenate is readily peptised by ferric chloride, ferric sulphate, or ferric nitrate. On dialysis, these colloids yield gels of excellent clearness and texture, except in the case of ferric sulphate, whereby a powdery coagulam is obtained.

The best method of the preparation of this gel is to coagulate the sol obtained by dialysing a mixture of ferric chloride in excess and potassium arsenate. Potassium arsenate when added to a solution of ferric chloride gives a yellowish white precipitate which dissolves on shaking, in the presence of the excess of ferric chloride. The addition of potassium arsenate is stopped when about three-quarters of the ferric chloride has been transformed to the arsenate. The solution at this stage is faint yellow in colour. The mixture is now allowed to dialyse for about a week. It gradually develops red colour as the process of dialysis proceeds on, which Holmes and Arnold rightly think to be due to the formation of ferric hydroxide sol by the hydrolysis of a little quantity of ferric chloride which was present there in excess. The sol, purified by dialysis, gives excellent transparent and stable jellies on the addition of electrolytes like potassium chloride or sulphate. The sol

[1] Loc. cit.

[2] Compt Rend *98* 1540 1884)

[3] J Amer Chem. Soc *40* 1014 (1918

is positively charged and not negative as Holmes[1] was led to think. If the dialysis is continued for a long time, the sol sets to a transparent jelly in the parchment dialyser Highly purified sols set themselves to jellies on ageing without the addition of foreign coagulating electrolytes. If the ferric chloride is not sufficient to peptise the whole of arsenate and still to remain in excess, the cuidy or opalescent jellies are obtained.

Chromic Arsenate Jellies.—Holmes[2] and co-workers have also prepared chromic arsenate jellies. The method of the preparation is exactly the same as was used in the case of ferric arsenate. A mixture of chromic chloride in excess and potassium arsenate ($KH_2 As O_4$) is dialysed for about a week and the clear greenish sol is coagulated by the addition of potassium chloride or sulphate, whereupon a clear transparent greenish gel is obtained. The jelly is very stable and can be preserved without undergoing any change. In the course of time, it acquires the vibrating property, and so does ferric arsenate jelly too. When sufficiently purified by dialysis, the sol sets to the jelly in the dialyser itself or in the bottle when allowed to age without the extra addition of coagulating electrolytes.

Mention has been made by Weiser[3] of the preparation of other arsenate jellies of cadmium, cobalt, aluminium, ferrous, etc., but the results are not much encouraging, and the jellies obtained are not fine in texture.

Thorium Arsenate Jellies.—Prakash and Dhar[4] have obtained for the first time the jellies of thorium arsenate. These jellies resemble manganese and zinc arsenates in their mode of preparation but differ from them in being slightly turbid and also in the fact that they require much higher concentration of arsenate solutions for the preparation.

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[1] J. Amer. Chem. Soc., *38*, 1972 (1916).
[2] Loc. cit.
[3] J Phys Chem *28* 96 (1924)
[4] J Indian Chem. Soc *6* 587 929

When to a thorium nitrate solution, a few drops of potassium arsenate solution are added a gelatinous precipitate appears which rapidly dissolves on shaking in the presence of an excess of thorium nitrate solution. The solution develops viscosity and finally the whole mass sets to an opalescent jelly. The best jellies of thorium arsenate are prepared by taking 5 c.c. of a solution of thorium nitrate (12·025 gms. in 250 c.c.) and adding to it 0·2 c.c. to 0·4 c.c. of 18 per cent potassium arsenate solution raised to 1 c.c. The mixture is shaken for about 2 minutes, and then allowed to set. The time of setting can be extended from that of a few minutes to about 24 hours by varying the concentrations of potassium arsenate.

These jellies are almost transparent with slight opalescence. In some cases the opalescence increases with time and the jellies ultimately become translucent or opaque. The jellies are very stable and do not undergo any syneresis.

Stannic Arsenate Jellies. —This jelly has also been for the first time prepared by Prakash and Dhar.[1] Stannic chloride solution when mixed with potassium arsenate solution gives the precipitate of stannic arsenate, but if stannic chloride be in excess, this precipitate dissolves and a clear colourless solution is obtained which on keeping develops opalescence and finally sets to an opalescent jelly on standing for some time.

Stannic arsenate jellies are opalescent or translucent at the time of formation, but they become opaque afterwards. The opacity increases more rapidly with the concentration of the potassium arsenate solution. However, the jellies are very stable, and do not undergo any marked syneresis.

The best stannic arsenate jellies are obtained by mixing 3 c.c. of M/1·099 stannic chloride solution with

[1] Loc cit

about 2 c c f 18 per cent potassium arsenate so ution keeping the total volume 6 c.c. The time of setting of the jelly varies with respect to the concentration of the reactants.

Ceric Arsenate Jellies.—This jelly has also for the first time been prepared by Prakash and Dhar.[1] When a solution of potassium arsenate is added to ceric ammonium nitrate solution in small quantities and allowed to stand, the yellow opalescence develops after a time, and under suitable concentrations the whole mass sets to a fine transparent or opaque jelly.

Some of these jellies are loose and in almost all the cases, they undergo syneresis within 12—24 hours, and finally break up. The best jellies are obtained by taking 4 c.c. of 10 per cent ceric ammonium nitrate solution and adding to it 0·2 to 0·4 c.c. of 18 per cent potassium arsenate solution keeping the total volume 4·5 c.c. The time of setting of this jelly cannot be extended beyond one hour.

Phosphate Jellies

Manganese and zinc do not appear to give phosphate jellies, though their arsenate jellies of the best texture are obtained with the least difficulty. The phosphate jellies so far prepared are of ferric iron, thorium and tin.

Ferric Phosphate Jellies.—Holmes and Rindfusz[2] were the first to observe that a sol of ferric phosphate sets to jellies when coagulated by the addition of electrolytes, or when dialysed for a long time. The mode of the preparation of ferric phosphate jelly is exactly the same as was used in the case of ferric arsenate jelly. To about a normal solution of ferric chloride is added potassium phosphate solution short of precipitation, the amount of ferric chloride remaining in excess. The mixture is now allowed to dialyse for about a week. A clear red sol is obtained,

[1] J Indian Chem. Soc., *7*, 367 (1930).

[2] J Amer Chem Soc *38* 1970 (1916)

which gives transparent stable jellies on coagulation with electrolytes as potassium chloride or sulphate. The sol when highly purified by dialysis also yields jellies on ageing by itself without adding any electrolyte.

Holmes and Arnold[1] have observed that a gel originating from the diammonium hydrogen phosphate (in a series of unwashed precipitates) sets in three days, the gel from the disodium salt in eight days and as might have been expected, that from a combination of these two, sodium ammonium hydrogen phosphate, in intermediate time, say five days. However, the best gels are obtained by the use of potassium dihydrogen phosphate.

Chromium and Aluminium Phosphate Jellies.—Holmes and Rindfusz (*loc. cit.*) have observed that similar to ferric phosphate jellies, aluminium and chromium phosphate jellies can also be prepared by coagulating their sols obtained by the dialysis of the mixtures of their chlorides or nitrates and potassium dihydrogen phosphate However, not much work has been done on these jellies. It appears that aluminium and chromium phosphates have less tendency of developing hydration, and consequently, their jellies are not so readily prepared as in the case of ferric phosphate.

Thorium Phosphate Jellies.—These jellies have been for the first time prepared by Prakash and Dhar.[2] They are prepared with the same ease as zinc and manganese arsenate jellies, and are amongst the most beautiful of the jellies so far prepared. They are perfectly transparent and free from opalescence, and so stable as could be kept as such for months without apparently undergoing any change or syneresis. The jellies are of the best texture and markedly elastic. The time of gelation in their case can easily be extended over a very long period by regulating

[1] J Amer. Chem. Soc., *40*, 1014 (1918).

[2] J Indian Chem Soc *6* 58 1929

the concentrations of the reactants These are the first phosphate jellies which have been prepared metathetically

5 c.c. of a solution of thorium nitrate (12·035 gms in 250 c.c.) are taken in test tubes and varying amounts of 22 per cent potassium phosphate solution (about 0·2 to 0·4 c.c.) are added, keeping the final volume 6 c.c. The mixtures are shaken well for about 3 minutes and then allowed to stand. The time of the setting of the jellies depends upon the concentration of potassium phosphate used. By regulating its concentration, the time of gelation can be extended from that of a few minutes to that of three days. Thorium phosphate jellies are so stable that they do not break or synerise in even months and may be preserved for over a year.

Stannic Phosphate Jellies.—This jelly has also been prepared for the first time by Prakash and Dhar.[1] It is prepared exactly in the same way as stannic arsenate jellies with which it resembles in every respect. The best stannic phosphate jellies are obtained by adding 1 to 3 c.c. of 22 per cent potassium phosphate solution (KH_2PO_4) to 3 c.c. of M/1·099 stannic chloride solution, and making the total volume 6 c.c. The transparent mixture so obtained develops opalescence and finally translucent or opaque jellies are obtained.

These jellies are also very stable and exhibit no marked syneresis. The opacity of these jellies increases on standing and finally, all the jellies become completely opaque.

Molybdate Jellies

No molybdate jellies had been previously prepared before we undertook the work. Prakash and Dhar[1] have for the first time prepared the molybdate jellies of iron, thorium, tin and zirconium.

Ferric Molybdate Jellies—When potassium molybdate solution is added to a ferric chloride solution, a yellowish white precipitate is obtained which dissolves on shaking if

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J Indian Chem Soc *6* 587 1929), *7* 367 1930)

ferric chloride is in excess. The clear mixture on standing for some time develops opalescence and finally, if the concentrations are suitable, the whole mixture sets to a firm opaque jelly.

Ferric molybdate jellies are obtained by adding varying amounts (3·5 to 5 c.c.) of 10 per cent potassium molybdate solution to 4 c.c. of M/2·69 ferric chloride solution, keeping the total volume to be 10 c c The clear mixture obtained by well-shaking the constituents sets to firm opaque jelly within a day or so.

Thorium Molybdate Jellies —When potassium molybdate solution is added to thorium nitrate, a white precipitate of thorium molybdate occurs, but if the mixture is vigorously shaken, the precipitate goes on dissolving till a clear viscous solution is obtained. This mixture on standing for some time, sets to a transparent colourless jelly. These jellies are very stable and do not synerise The best of the thorium molybdate jellies are prepared by adding about 0·3 to 0·8 c.c. of 10 per cent potassium molybdate solution to 5 c c. of thorium nitrate solution (12·035 gms. salt in 250 c.c.) making the total volume 6 c.c., and allowing the mixture to stand for 5 minutes or so. Some of the molybdate jellies prepared by the use of comparatively higher concentrations of potassium molybdate break up in the course of 10—12 days leaving a white powder

Stannic Molybdate Jellies.—No jelly has been obtained by directly mixing the solutions of potassium molybdate and stannic chloride whereby a white precipitate of gelatinous nature is only formed. However, if potassium molybdate (15 per cent solution) beadded below precipitation value to stannic chloride solution and the mixture is allowed to dialyse for 24 hours, a clear sol with slight opalescence is obtained. The sol is fairly stable and sets to translucent firm jellies on the addition of coagulating electrolytes like potassium chloride or sulphate Like other stannic jellies

these are almost transparent though accompanied with slight opalescence, at the time of formation, but become opaque on keeping for some time.

Zirconium Molybdate Jellies.—When potassium molybdate solution is added to zirconium nitrate solution, a white precipitate of zirconium molybdate is obtained which is easily dissolved by an excess of zirconium nitrate on shaking. In this way, a sufficient amount of zirconium molybdate can be peptised and a concentrated sol obtained.

This sol on dialysis gives suitable jellies when coagulated with electrolytes like potassium chloride or sulphate. If the dialysis were carried for a long time, the sol either sets on the parchment paper or gives a transparent jelly on standing for some days without the addition of electrolytes

Some of the jellies prepared by the coagulation of the sol by potassium chloride develop opalescence and may even become opaque.

A sol prepared by adding 10 per cent solution of potassium molybdate to 70 c.c. of M/1·33 zirconium nitrate till the precipitate obtained just dissolved in excess of zirconium nitrate and dialysed for 36 hours gave good jellies with N-KCl or N/5 potassium sulphate. Strength of the sol was 50·3 gm. zirconium molybdate per litre.

Tungstate Jellies

No tungstate jelly has ever been prepared before. Prakash and Dhar[1] have prepared ferric tungstate, chromic tungstate, stannic tungstate and thorium tungstate jellies. No jellies have yet been prepared of the tungstate of ceric or zirconium.

Ferric Tungstate Jellies.—Ferric tungstate jellies have been obtained by two methods: firstly, by directly mixing ferric chloride with sodium tungstate, and secondly, by dialysing and coagulating the sol obtained by peptising ferric tungstate with excess of ferric chloride.

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When 15 per cent solution of sodium tungstate is added to M/2 ferric chloride, a bulky precipitate is formed which dissolves to some extent on vigorous shaking. If the mixture be warmed in a water-bath at 96°C. for 5 minutes, it is completely dissolved, and a clear transparent yellow solution is obtained which sets to fine yellow opaque jelly when the concentrations are suitable.

A yellow opaque jelly is obtained by mixing 2 c.c. of M/2 ferric chloride with 3 c.c. of 15 per cent sodium tungstate, and warming the mixture for 5 minutes at 95°C. These jellies resemble ferric molybdate jellies and are very stable.

More transparent jellies are obtained when a mixture of sodium tungstate and ferric chloride in excess is dialysed and the sol thus obtained is coagulated with electrolytes To 75 c.c. of 0·929 M ferric chloride solution was added a solution of 16 5 gms. sodium tungstate ($Na_2WO_4 . 2H_2O$) with vigorous shaking and slight warming and the volume was made up to a litre. The mixture was filtered and dialysed for six days. The clear sol thus obtained contained 21·68 gms. ferric tungstate per litre and set to translucent jellies when coagulated with N-KCl or N/20 K_2SO_4.

Chromic Tungstate Jellies.—When sodium tungstate solution is added to chromic chloride solution, a bulky greenish white precipitate is formed, which slightly dissolves on shaking at the room temperature (25°—30°C.) even when chromic chloride is present in large excess. However, if the mixture is warmed and shaken, a sufficient amount of chromic tungstate is peptised and it forms a clear transparent solution. If this mixture is now allowed to dialyse for about ten days, a translucent sol is obtained which sets to a jelly on the addition of potassium sulphate.

To M/1·5 chromic chloride solution, a 15 per cent solution of sodium tungstate was added, so long as the precipitate obtained could be re-dissolved on warming The

solution still contained some of the free ferric chloride and it was dialysed for ten days, when a sol containing 23·86 gms chromic tungstate per litre was obtained.

Firm opalescent jellies were obtained by coagulating this sol with N/50 potassium sulphate The period of setting could be extended to even two days The sol set itself to a gel when kept in a jena glass vessel for about a month

Stannic Tungstate Jellies —When stannic chloride solution is mixed with sodium tungstate and if there is not much of the free hydrochloric acid in the stannic chloride solution, the clear mixture develops opalescence and after some time, stable opaque Jellies of stannic tungstate are obtained.

2 c.c of 1 5.3 M stannic chloride solution were taken in test tubes and to it varying concentrations (3-4 c.c.) of 15 per cent sodium tungstate were added. The volume was made up to 6 c.c. The mixture was thoroughly shaken for 5 minutes, when a turbid solution was obtained. The solution became quite clear when kept for another 10 minutes After some period, which differs according to the concentrations of the tungstate, slight opalescence begins which intensifies and finally, an opaque jelly is obtained.

The time of setting of these jellies can be extended from that of an hour to two days All these jellies are opaque, white, hard and stable, and do not undergo any marked syneresis.

Thorium Tungstate Jellies.—When sodium tungstate is mixed with thorium nitrate solution, a white precipitate occurs, and no jelly is formed. Many concentrations have been tried but to no success.

However, if to 4 c c. thorium nitrate solution (12·06 gms. litre) about 0·5-1 c c. of glycerine be added and then a few drops of 5 per cent sodium tungstate solution, the mixture at once becomes very viscous and in a short time

it sets to a transparent stable and firm jelly which does not undergo any marked syneresis, when kept for a number of days.

Borate Jellies

No work appears to have been done on the borate jellies before we undertook the investigations Prakash and Dhar[1] have prepared the ferric, stannic, zirconium and ceric borate jellies for the first time.

Ferric Borate Jellies. When a saturated solution of borax is gradually added to ferric chloride solution, a bulky precipitate occurs which goes on dissolving on shaking, but when sufficient quantity of borax has been added, the ferric borate settles down suddenly in the form of a bulky opaque jelly, which begins to syneirse soon and then it breaks up

However, very transparent jellies of ferric borate, similar to arsenate and phosphate, are obtained by dialysing a mixture of 60 c.c. of ferric chloride (M/2 69) and 45 c c. of 20 per cent borax, containing an excess of ferric chloride to peptise ferric borate. The clear red solution thus obtained was allowed to dialyse for thirty days The concentration of the sol was 11·78 gms. ferric borate per litre. It set to transparent stable red jellies when coagulated with N-potassium chloride or N/50 potassium sulphate.

Stannic Borate Jellies.—When a saturated borax solution obtained at 90°C. is added to 1 5 M stannic chloride solution, a white precipitate of stannic borate is formed which dissolves on shaking. If suitable concentrations are chosen and the mixture warmed at 90—95°C a loose white opaque jelly is obtained which breaks up in a short time. If stannic chloride contains much free hydrochloric acid, which is always associated with hydrated stannic chlorides, stannic borate does not set even on warming

To 1 5 M stannic chloride solution, a saturated borax solution is added, and the mixture containing an excess

[1] Loc cit

of stannic oxide was dialysed for 24 hours. A clear transparent sol containing 66·4 gms SnO_2 per litre was obtained which set to transparent jellies with slight opalescence when coagulated by the addition of electrolytes potassium chloride or sulphate. The sol itself becomes more and more viscous even without the addition of electrolytes and sets within 24 hours.

Zirconium Borate Jellies.—Zirconium borate jellies are obtained in the same way as zirconium molybdate jellies. A hot concentrated solution of borax was added to 70 c.c. of M/1·33 zirconium nitrate solution till the precipitate of zirconium borate obtained just dissolved in the excess of zirconium nitrate. The solution was dialysed for three days. The concentration of the sol thus obtained was 34·62 gms zirconium borate per litre. It gave transparent or opalescent jellies when coagulated by electrolytes, like N-KCl or N/5 K_2SO_4.

It has been observed in the case of both zirconium molybdate and borate jellies that they are readily obtained when their sols are coagulated by potassium chloride but these jellies develop opalescence on standing. However, when the sol is coagulated by potassium sulphate, the jellies are more transparent and do not develop opalescence. The jellies are very stable, and if the coagulating electrolyte is not in much excess, they do not undergo any marked syneresis.

Ceric Borate Jellies.—When a solution of borax is added to a solution of ceric ammonium nitrate, a yellowish white is obtained which readily dissolves on shaking, if the ceric ammonium nitrate is in excess. If the mixture of the two substances is allowed to stand for some time, the contents are generally precipitated, though in some cases loose unstable jellies may also form.

The clear solution formed by mixing 100 c.c. of 10 per cent ceric ammonium nitrate and 35 c.c. of 15 per cent

borax solution was dialysed for 24 hours The sol thus obtained not only gave a jelly on treatment with N/20 potassium sulphate solution, but also on keeping for some time in a jena glass bottle, its viscosity increased continuously and in the next 24 hours, it set completely to an opalescent jelly.

Another sol was prepared by mixing only 30 c.c. of 15 per cent borax solution to 100 c.c. of 10 per cent ceric ammonium nitrate. The mixture on dialysis gave a clear sol in the course of 24 hours. The sol was quite stable and gave stable jellies on the addition of electrolytes.

Sulphide Jellies

The sulphide sols are more or less hydrophobic when compared to the sols of hydrous oxides. They do not appear to develop hydration tendency which is so essential for the formation of jellies. Any record of the formation of sulphide jellies is of Usher,[1] who appears to have gelatinised cadmium sulphide in the presence of suitable concentrations of sodium chloride. This method he also employed for the preparation of gamboge jellies.

Usher prepared a sol of cadmium sulphide by passing hydrogen sulphide through the thoroughly washed precipitate suspended in water. This sol was treated with varying quantities of sodium chloride and it was found to gelatinise if the concentration was between N/100 and N/10. Thus in a mixture in which the final concentration of cadmium sulphide was 1·51 per cent by volume, decinormal sodium chloride caused gelatinisation immediately ; twenty-fifth normal in two minutes and fiftieth normal after several hours.

Von Weimarn Jellies

Von Weimarn has done a sort of pioneering work in the field of precipitation, and his laws in this connection have

[1] Proc. Roy. Soc., A., *125*, 143 (1929).

ie e ved a wari approval in certain quarters He has become successful in obtaining gelatinous precipitates from such substances which under ordinary conditions are incapable of developing hydration The most important of his jellies is of barium sulphate A saturated solution of barium thiocyanate when mixed with a saturated solution of manganous sulphate gives gelatinous precipitate or even a transparent jelly if the concentrations are fairly regulated. At lower dilutions of the above solutions, only granular precipitates are obtained.

For the preparation of Weimarn-jellies, the highly concentrated solutions are required In the case of barium sulphate jelly, we have found that barium acetate may be used with almost an equal success Kato[1] obtained stable gels by the interaction of sulphuric acid and barium acetate in an alcohol water mixture in which barium sulphate is more soluble than in water alone, thus developing a higher degree of supersaturation. Similarly, Lenher and Taylor[2] prepared stable gels by the interaction of dilute solutions of barium chloride and sulphuric acid in selenium oxychloride, in which barium sulphate is almost entirely insoluble.

Kato also observed that barium sulphate precipitated from aqueous alcohol comes down as a gel which forms a clear sol on shaking with a larger quantity of water. Strong adsorption of the alcohol, he thinks, undoubtedly helps to prevent coalescence or growth of the colloidal particles, since a jelly formed from the aqueous solution does not form a stable sol on shaking with water.

The jellies of various other sparingly soluble salts have also been prepared by the interaction of the concentrated reactants. Buchner and Kalff[3] have given the following table in which the values of N, which is the ratio between

[1] Mem Coll Sci Kyoto Imp Univ., *2*, 187 (1909).
[2] J. Phys Chem., *28*, 962 (1924).
[3] Rec Trav Chim *39* 135 (1920

ant of supersaturation P, and solubility of the

$$N = P/L$$

ature of the precipitate have been illustrated.

L	N	State of precipitate
4×10^{-4}	3,400	Jelly.
18×10^{-3}	75	Jelly
3×10^{-2}	140	Jelly.
2×10^{-5}	100,000	Jelly
1×10^{-5}	700,000	Colloidal, unstable
7×10^{-7}	8,000,000	Colloidal, unstable
$1{\cdot}5 \times 10^{-8}$	30,000,000	Jelly, unstable
$4{\cdot}8 \times 10^{-3}$	360	Colloidal.

is been supposed by many authors that in the setting , the first stage is of the formation of a gel. Cavazzi[1] ube[2] for independent reasons suggest that the set- this substance consists in the initial formation of a h gradually changes into needle-shaped crystals of Cavazzi bases his suggestion on the observation sum can be precipitated under certain conditions, gel in which small crystal needles are subsequently

ium Chloride Jelly.—Michael[3] observed that sodium formed by the interaction of sodium malonic ester racetic ester in dry benzene is stabilised in the sol by strong adsorption of one or more organic . It has been later observed that sodium chloride by the interaction of sodium ethylate or sodium ate and hydrochloric acid in a mixture of ether and ohol, is a jelly or gelatinous precipitate.

z. Chim. Ital., *42*, (2) 626 (1912); Kolloid-Z., *12*, 196 (1913).
oid-Z., *25*, 62 (1919).
, *38*, 3217 (1905)

Calcium acetate jelly Baskerville observed that if 80 cc of 95 per cent alcohol be mixed with 15 cc of the saturated solution of calcium acetate in water, the whole mass of the alcohol sets to a fine solid transparent jelly. The jelly undergoes syneresis in a short time The alcohol of the jelly can be replaced exactly in the same manner by acetone.

Recently, Thorne and Smith[2] have studied this jelly in details. They prepared the jelly by pouring a saturated aqueous solution of calcium acetate into alcohol. Most of the gels thus prepared are not stable for more than 24 hours: they are opalescent at first but gradually soften with time. The stability of the gels is increased in some cases to six months, by the addition of acetone or various oleates. Gels containing sodium oleate exhibit syneresis. These investigators have also studied the influence of temperature, and various ions on the stability of these jellies.

[1] U. S. P 1208265 (1916).
[2] Kolloid-Z., *48*, 113 (1929).

DETECTION OF IRON, THALLIUM, TITANIUM AND ZIRCONIUM IN A MIXTURE

BY

I. K. TAIMNI

The analysis of mixtures containing only common elements can be carried out without much difficulty, because as a result of extensive investigations of the analytical properties of these elements we have now at our disposal fairly satisfactory schemes for their separation and detection. But the separation and detection of the rarer elements is still attended with considerable difficulty as the properties of these elements have not yet been sufficiently investigated for the elaboration of methods which are simple as well as accurate. Some useful schemes have, however, been devised for the analysis of mixtures containing both common and the rarer elements. The work of Noyes, Bray and Spear in this field is especially valuable. The scheme of qualitative analysis devised by them [J.A.C.S., 29, 137 (1907) and 30, 481 (1908)] can be taken as a good basis for work in the qualitative analysis of mixtures containing the common elements as well as the more important of the rarer elements. There are, however, a few manipulative operations in their scheme which render its adoption in ordinary laboratory work difficult if not impossible. It is true that the difficulties encountered in the detection of the rarer elements are so great that the inclusion in any scheme of unusual and rather inconvenient methods may be quite justified. But the more these methods are replaced by others which are simpler and more convenient, the easier it will be to adopt such schemes in ordinary class work. Under certain circumstances even some sacrifice of analytical accuracy may be justified where, for instance an extremely sensitive but

inconvenient method for detecting an element is replaced by a less sensitive but more convenient method. A manipulative operation in the scheme put forward by Noyes, Bray and Spears which makes it unsuitable for ordinary class work is the separation of iron and thallium from zirconium and titanium by means of ether. At one stage in the analysis these four metals are precipitated together in the form of hydroxides, basic acetates, or phosphates. The precipitate is dissolved in HCl of a definite concentration and the solution is shaken with ether in a separating funnel. The chlorides of iron and thallium pass into the ethereal layer, while all of the zirconium and titanium remains in the aqueous layer. By repeating the operation twice or thrice all the iron and thallium can be dissolved out from the aqueous solution. As far as the effectiveness of the operation is concerned it is undoubtedly an excellent method of separating these metals, but it needs hardly be pointed out that the method is neither cheap nor convenient, especially in the hot weather of India where the temperature in most places is above 100°F. For these reasons the author had been for some time trying to devise a method of detecting these four metals in the mixed precipitate without the use of ether. On studying the methods of identifying these four elements it was found that the very scheme given by the authors could be modified in such a manner as to eliminate the use of ether without necessitating any sacrifice of analytical accuracy. In the method recommended by Noyes, after the separation of iron and thallium from zirconium and titanium, thallium is identified by precipitation as thallous iodide with potassium iodide and sulphurous acid, iron by the usual thiocyanate test, zirconium by precipitation as phosphate with sodium phosphate in presence of sulphuric acid, and titanium by conversion into salt of TiO_3 with H_2O_2 in presence of sulphuric acid.

Now, if the specific test for each of the four elements can be applied in presence of the remaining three

it will obviously simplify the whole procedure if the mixed precipitate were dissolved in sulphuric acid and small portions of the solution so obtained were tested for each of the four elements that may be present. A study of these tests showed that it is possible to test for each of the elements in a portion of the solution obtained with sulphuric acid without any interference from the other elements provided the concentration of sulphuric acid is properly regulated. Before dealing with the procedure to be adopted the individual tests may be discussed.

(*i*) *The Test for Thallium.*—In order to determine the sensitiveness of the test for thallium with potassium iodide and sulphurous acid, a solution of thallic chloride (containing 0·1 mgm. thallium per cc of the solution) was added from a burette to mixtures of potassium iodide and sulphurous acid containing varying quantities of sulphuric acid, and the points at which distinct precipitate of thallous iodide appeared were determined. The following results were obtained.

TABLE I

Showing the effect of varying the concentration of H_2SO_4 on precipitation of TlI

1N KI=1 cc.
0·6N H_2SO_3=5 cc.
H_2SO_4=10 cc
Total volume=20 cc

	10N H_2SO_4	5N H_2SO_4	1N H_2SO_4	0N H_2SO_4
05 mgm Tl.	No precipitate	No precipitate	No precipitate	No precipitate
0·1 mgm. Tl.	No precipitate	No precipitate	No precipitate	No precipitate
0·2 mgm. Tl.	Opalescence	Opalescence	Opalescence	Opalescence
0·3 mgm. Tl	Distinct precipitate	Distinct precipitate	Distinct precipitate	Distinct precipitate

From the tables given above it will be seen that 0·1 mgm Tl can be detected in a volume of 10 cc and the quantity of H_2SO_4 up to a concentration of 5N has no appreciable effect on the sensitiveness of the test. Since the other three metals do not give a precipitate with KI in presence of sulphuric acid the presence of thallium can be detected by taking a small portion of the solution in sulphuric acid and treating it with KI and H_2SO_3. A test analysis with 100 mgm each of Ferric iron, zirconium and titanium and 0·1 mgm of Tl showed that a small amount of thallium can be easily detected in presence of a large excess of the other metals (In the test analyses the metals were precipitated together as hydroxides with NH_4OH, the precipitate was dissolved in sulphuric acid and the solution tested for the metal present in small quantity by the specific test)

(*ii*) *The Test for Iron.*—The delicacy of the thiocyanate test for iron is well known Even 0·01 mgm. Fe in 10 cc. of the solution can be easily detected Since the other three metals do not give any colour or precipitate in presence of sulphuric acid, a portion of the solution in sulphuric acid can be tested for iron by means of potassium thiocyanate. Of course, if it is necessary to add ferric chloride for the separation of phosphate from metals of the alkaline earth group, the test for iron should be performed before the solution is treated with ferric chloride solution It may be mentioned here that on the addition of a potassium thiocyanate to a solution containing thallic salt and sulphuric acid a yellow colour appears but this colour fades very quickly and there is no difficulty in detecting the presence of iron. A test analysis with 100 mgm. each of thallium, titanium and zirconium and 0·1 mgm. iron showed that it is easy to detect even a trace of iron in presence of a large excess of the other metals.

(*iii*) *The Test for Titanium.*—It might be imagined that the presence of iron in the mixed precipitate will hinder

the detection of small quantities of titanium on account of the yellow colour of ferric salts. As a matter of fact, it is not at all difficult to detect even a trace of titanium in presence of a large quantity of ferric salt because dilute ferric sulphate solutions in presence of sulphuric acid are practically colourless and no colour is developed even on treating the solutions with H_2O_2. If, therefore, the mixed precipitate of the hydroxides is dissolved in sulphuric acid and a portion treated with H_2O_2 even a trace of titanium will be easily detected by the appearance of a yellow colour. Should the solution before the addition of H_2O_2 have a slightly yellow colour, owing to the presence of a very large quantity of iron, all that is necessary is to dilute a portion of the solution sufficiently, so that the colour of ferric salt is almost inappreciable. As the H_2O_2 test for titanium is extremely delicate this dilution does not in any way hinder the detection of even small quantities of titanium. A test analysis with 100 mgm. each of iron, zirconium and thallium and 0·1 mgm. titanium showed that quantities of this element even smaller than 0·1 mgm. can be easily detected in presence of a large quantity of iron. An additional blank test was performed with 100 mgm. iron and H_2O_2 in presence of sulphuric acid, when no change in colour was observed. In view of the extremely delicate nature of the H_2O_2 test for titanium it appears superfluous to precipitate the titanium again as phosphate by adding sodium phosphate to the acid solutions. The phosphate test is decidedly less delicate and less distinctive on account of the similar precipitation of zirconium phosphate. The presence of a large excess of sulphuric acid does not interfere with the H_2O_2 test while it does hinder the precipitation of titanium as phosphate.

(*iv*) *The Test for Zirconium.*—The precipitation of the phosphate in presence of a large excess of sulphuric acid is at present the most characteristic and reliable test for

zirconium No other metal except titanium gives a precipitate with sodium phosphate under this condition, but if the titanium is previously oxidized to the hexavalent condition by means of H_2O_2 the phosphate test is specific for zirconium According to Noyes, a large excess of sulphuric acid hinders the precipitation of small quantities of zirconium. He found that when the concentration of sulphuric acid is 1·5N quantities of zirconium less than 1 mgm. are precipitated after about an hour, and with a greater concentration of H_2SO_4 even larger quantities may not be precipitated. Since in the quantitative estimation of zirconium by precipitation as phosphate the addition of even 20 per cent by weight of H_2SO_4 is advised (Treadwell, Analytical Chemistry, 1924, Vol. II. p. 123), it was considered worth while to investigate the effect of varying the concentration of sulphuric acid on the precipitation of zirconium phosphate.

Quantity of zirconium in solution.	1 10 c c. 10% Na_2HPO_4 10N H_2SO_4.	2 10 c c 10% Na_2HPO_4 5N H_2SO_4	3 10 c c. 10% Na_2HPO_4 2N H_2SO_4	4 10 c c 10% Na_2HPO_4 1N H_2SO_4	5 10 c c 10% Na_2HPO_4 0·5N H_2SO_4.	10 c c 10% Na_2HPO_4 0·1N H_2SO_4.
0·1 and 0·2 mgm. ..	No opalescence	No opalescence.	No opalescence.	No opalescence	No opalescence.	No opalescence
0·3 and 0·4 mgm. .	Opalescence	Opalescence	Opalescence	Opalescence	Opalescence	Opalescence
0·5 mgm. ..	Slight precipitate (finely divided)	Slight precipitate (finely divided)	Opalescence	Opalescence	Opalescence	Slight precipitate (finely divided)
0·6 mgm. .	Distinct precipitate	Distinct precipitate.	Distinct precipitate.	Opalescence	Opalescence	Distinct precipitate.
0·7—2·5 mgm. ...	..	...		Opalescence	Opalescence	.
3—4 mgm. ...	...	..	...	Precipitate (finely divided).	Opalescence	
5 mgm. ...	...	.			Precipitate (finely divided).	...

TABLE III

Showing the effect of varying the concentration of sulphuric acid on the precipitation of zirconium phosphate from hot solution.

Quantity of zirconium in solution.	1 10 c c. 10% Na_2HPO_4 10N H_2SO_4.	2 10 c c 10% Na_2HPO_4 5N H_2SO_4.	3 10 c.c 10% Na_2HPO_4 2N H_2SO_4	4 10 c.c 10% Na_2HPO_4 1N H_2SO_4.	5 10 c c 10% Na HPO_4. 0·5N H_2SO_4.	6 10 c c 10% Na_2HPO 0·1N H SO
0 1 mgm	No opalescence	No opalescence.	No opalescence.	No opalescence.	No opalescence	No opalescence
0 2 mgm.	Clear flocculent precipitate.	Clear flocculent precipitate	Clear flocculent precipitate.	No opalescence	No opalescence.	Slight precipitate
0 3 mgm ...	.	...		Opalescence	Opalescence	Clear flocculent precipitate
0 4 mgm. ...	.	...	...	Slight precipitate	Opalescence	

A standard solution of zirconium oxychloride (containing 1 mgm. zirconium per c.c. of solution) was added from a burette 0·1 c.c. at a time, to cold mixtures of 10 c.c. of 10 per cent sodium phosphate and 10 c.c. solutions of sulphuric acid of different concentrations. The solutions were thoroughly shaken and allowed to stand for about 10 minutes after each addition of 0 1 c.c. zirconium solution. The results are shown in Table II. Since heating the solutions were found to accelerate the precipitation of zirconium phosphate, all the above experiments were repeated with this difference, that all the solutions after every addition of 0·1 c.c zirconium oxychloride solution were heated to 60°—80°. The results are shown in Table III.

From tables II and III the following facts are apparent:

(*i*) A precipitate appears with much smaller quantity of zirconium when the solutions are heated than when they are allowed to remain cold. In the former case, the minimum quantity of zirconium which gives a precipitate in about 10 minutes is 0·2 mgm. while in the latter case it is 0·5 mgm.

(*ii*) When the solution is heated the precipitate is obtained in a flocculent condition, while it is more or less finely divided and difficult to detect in small quantity, when obtained in the cold.

(*iii*) The quantity of sulphuric acid in solution does not make much difference except when the concentration of sulphuric acid lies near about 0·5 N—0·25 N. It will be seen from the tables that the precipitate appears for the first time with practically the same quantity of zirconium in mixtures containing 5N, 2·5N, 1N, 0·05N H_2SO_4 but a much larger quantity of zirconium has to be added before a precipitate appears in solutions containing 0 5N and 0 25N H_2SO_4

This retarding influence of sulphuric acid at this concentration was repeatedly verified, and makes it necessary to keep the concentration above 1N, if small quantities of zirconium are to be detected.

It will be seen from the above that the phosphate test for zirconium is sufficiently sensitive for qualitative purposes even in presence of large quantities of sulphuric acid provided the solution is heated after the addition of sodium phosphate. It has already been shown by a number of authors that it is necessary to add a large excess of a soluble phosphate to completely precipitate small quantities of zirconium. Since the red compound formed by titanium and H_2O_2 does not decompose in presence of a large excess of sulphuric acid or on heating to 60°—80° there is no danger of the precipitation of titanium phosphate under these conditions.

The phosphate test is also very sensitive when the concentration of sulphuric acid is 0·05N or lower, but it is not permissible to use such low concentrations on account of the danger of precipitation of ferric or thallic phosphates. In order to determine the minimum limit of H_2SO_4 concentration which will keep these two phosphates in solution mixture of 10 c.c. 10 per cent Na_2HPO_4 and 10 c.c. H_2SO_4 solutions of different concentrations were treated with ferric and thallic salts with the results shown in Table IV.

TABLE IV

	10 c.c. 10% Na_2HPO_4, 10 c.c. 0·1N H_2SO_4	10 c.c. 10% Na_2HPO_4, 10 c.c. 0·5N H_2SO_4	10 c.c. 10% Na_2HPO_4, 10 c.c. 1N H_2SO_4
100 mgm Fe	Yellow precipitate	White precipitate.	No precipitate
100 mgm Tl	Yellow precipitate	No precipitate	No precipitate

From the above results we see that when the solution is 0·5N with respect to H_2SO_4 and contains 100 mgm. either of iron or thallium, neither ferric phosphate nor thallic phosphate is precipitated If therefore the concentration of sulphuric acid is above 1N there is no danger of the precipitation of either Fe of Tl as phosphate

On making a test analysis with 100 mgm each of thallium, iron, and titanium and 0·5 mgm. zirconium, no precipitate of zirconium phosphate was obtained even in an hour, although the quantity of zirconium was more than sufficient to give a precipitate according to results given in Table III. By making test analyses, combining only one metal in large quantity with a small quantity of zirconium it was found that it was iron which hindered the immediate precipitation of zirconium in small quantities In presence of 100 mgm. iron even 1 mgm. of zirconium is not immediately precipitated though in its absence 0·5 mgm. zirconium gives a clear flocculent precipitate. A precipitate is, however, obtained with 1·5 mgm. zirconium even in presence of 100 mgm. iron. In presence of iron, the phosphate test for zirconium is rendered slightly less delicate but it is sufficiently delicate for qualitative purposes in ordinary class work.

From a consideration of the tests discussed above the following procedure for the analysis of the precipitate containing the hydroxides of the four metals may be devised :—

Treat the precipitate of the hydroxides with about 5N H_2SO_4 solution until it just dissolves. Then add an equal volume of 2·5N H_2SO_4, and filter the solution if it is not quite clear. In this way a solution will be obtained with a normality lying between 2·5N and 1·25N. The object should be to keep the total volume of the solution as small as possible and if the quantity of the precipitate is very large sulphuric acid with a normality greater than

5N should be used for the preliminary neutralization of the hydroxides

(*a*) To a small portion of the solution (about 2 c.c.) add 1 c.c. 1N KI solution and 5 c.c. saturated SO_2 solution. The formation of a yellow precipitate proves the presence of thallium. A yellow colour alone does not indicate the presence of thallium, because when KI and H_2SO_3 solutions are mixed, a yellow colour is obtained.

(*b*) To another small portion of the solution (about 2 c.c) add 5 c c. 1N KCNS solution a blood-red colour shows the presence of iron Since this is an extremely delicate test for iron if a light red colour is obtained, a blank test should be performed with the acids used in the previous procedures, to see whether these are contaminated with traces of iron. If it is necessary to add ferric chloride for the separation of phosphoric acid from metals of the alkaline earth group, iron should have been tested for before the addition of ferric chloride solution.

(*c*) To the remaining portion of the solution add 5—10 c.c. 3 per cent H_2O_2 solution. A yellow to orange colour indicates the presence of titanium. If the solution before the addition of H_2O_2 is slightly yellow owing to the presence of a large quantity of iron, dilute it with water till the colour is almost inappreciable.

(*d*) To the solution which has been tested for with H_2O_2, add 5 c.c. 10 per cent Na_2PHO_4 solution. Heat to about 70°— 80°. A white flocculent precipitate proves the presence of zirconium. As very small quantities of zirconium (less than 1 mgm.) are precipitated slowly, the solution should be examined again after about an hour to see if a slight precipitate has separated during this time.

The procedure is practically the same when phosphoric acid is removed from the solution by means of ferric chloride in presence of sodium acetate and acetic acid, and the mixed precipitate consists of phosphates basic acetates

or hydroxides of the four elements but the following points should be borne in mind in this case

(*i*) Iron should be tested for in the usual way before adding ferric chloride for elimination of phosphoric acid from the solution.

(*ii*) Zirconium and titanium are not likely to be present since the phosphates of these metals are insoluble in dilute mineral acids.

CHEMICAL EXAMINATION OF THE KERNELS OF THE FRUIT OF THEVETIA NERIFOLIA (JUSS)

BY

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Thevetia nerifolia or yellow oleander as it is known in English and Pila-kaner in Hindustani is a plant of the natural order Apocynaceae, commonly cultivated in India as an ornamental garden shrub. The fresh bark of the young wood, of from ½--1 inch in diameter, is green, smooth and covered by a thin grey epidermis, through which the green colour is apparent; it turns black when dry. All parts of the plant yield an abundance of acrid milky juice. The flowers are yellow. The fruit is globular, slightly fleshy, green, 1 to 2 inches in diameter, and contains a hard nut, light brown in colour and triangular with a deep groove along the edge corresponding to the base of the triangle, each nut contains two pale yellow, slightly winged seeds with a light brown coating.

Descourtilz, in his "Flora of the Antilles," speaks of Thevetia nerifolia as an acrid poison, of the bark as a drastic purgative, of the fruit as emetic and of an extract of the plant as a remedy for intermittent fever. He describes the case of a young negro who had eaten of the green fruit, and who was affected with chills, delirium, and other nervous symptoms, nausea and a thready pulse; he had irregular spasms, followed by extreme agitation, with singing laughing and weeping and then followed by a

fixed blank look He seemed tending to coma but was relieved by an emetic

The kernels of the fruit are extremely bitter and when chewed produce a slight feeling of numbness and heat in the tongue De Vrij (Pharm. J Trans., 3, *12*, 457) very many years ago discovered in the seeds a glucoside which was closely studied by Blas. Blas and T. Husemann (Archiv. für exp. pathol. U. Pharm., V. 228) tested the active principles of the plant thevetin and theveresin on animals. The former has upon frogs the same effects as digitalin, and the lethal dose is also nearly the same (gm. 0·001-0·003). Experiments upon dogs and rabbits led these investigators to recognise a strong analogy between the effects of these glucosides and the effects of digitalin and helleborin and other analogous products.

Dr. Dumontier has published an account of the death of a child three years old after eating one seed. An interesting case of poisoning by one of the seeds is recorded by Dr. J. Balfour (Madras Journal of Lit. and Sciences, III, N. Ser., p. 140) Dr. Lyon (Med. Jouris., p. 299) remarks that cases of poisoning in the human subject are seldom met with in India, but of late years the seeds have come into somewhat extensive use in Bombay Presidency as a cattle poison. In Bengal four cases are on record, but the particulars of one only are given, in which a woman attempted to commit suicide.

According to De Vrij (loc. cit.) the kernels of the seeds give with benzine 57 per cent of a limpid, almost colourless oil. Bhattacharya and Ayyar (Journal of the Indian Institute of Science, Vol. XA, *2*, 15) also obtained with light petroleum a pale yellow oil, the oil content being 57 per cent. But it has been possible for the present investigator to obtain 68·6 per cent of a pale yellow, non-drying oil on careful and exhaustive extraction of the crushed kernels with petroleum ether (B P 35 60°C

Blas (U. S. A. Pharm , 1918, 1639) gave the formula $C_{54}H_{84}O_{24}$ to thevetin and $C_{48}H_{70}O_{17}$ to theveresin. He confused thevetin with cerberin, previously found by Oudemann in Cerbera odallam Ham., which was afterwards refuted by Plugge (A. Pharm., 1893, 10).

Warden (Pharm. J Trans., 3, *12*, 417-18) isolated thevetin by alcoholic extraction of the oil free kernels by concentrating the extract. From the filtrate, after a course of chemical treatment, he got pseudo-indican contaminated with thevetin and extractives. He seemed to have isolated a second glucoside (loc. cit.) by precipitating the mother liquor left after the crystallization of thevetin, by aqueous tannic acid and decomposing the precipitate by lime.

R. Weitz and A. Bonlay (Bul. Sci. Pharmocol., 1923, *30*, 81—88) also extracted a bitter principle from the kernels which gave with H_2SO_4 an orche-yellow coloration becoming pink after 12 hours.

The above represents the work that has hitherto been done on the kernels of the fruit of Thevetia nerifolia. None of the authors gave any definite properties for identification of the products they isolated. Few colour reactions have been given which are, of course, easy to study in cases where the isolation of chemically pure compounds is a matter of considerable difficulty.

The kernels, which were 22·6 per cent of the dry nuts, gave 68·5 per cent of a straw yellow non-drying oil. The kernels on further extraction with 97 per cent ethyl alcohol gave a brown syrupy liquid which on distillation over water-bath under vacuum (8 mm.) was obtained as a light brown solid mass. This on extraction with chloroform gave about 3 per cent (of the kernels) a light yellow solid mass completely soluble in ethyl acetate. On crystallization from ethyl alcohol and animal charcoal it was obtained as fine snow white needles melting at 194°C. This was the water insoluble glucoside. This product has been named

Thevetosin' by the present author It was optically active having a positive rotation

After removing thevetosin from the alcoholic extract it was completely freed from chloroform. A lump of light yellowish brown mass was obtained. It was very hygroscopic and contained a glucoside very soluble in water, glucose and other water-soluble impurities. This glucoside was "thevetin," the active principle of the kernels, which on hydrolysis with mineral acids gave Warden's (loc. cit.) thevetin-blue Thevetidine, the genin of thevetin, separates readily on hydrolysis as a light brown oily liquid. It very soon gets oxidised changing colour to greenish blue, blue and finally into a black mass. It is little soluble in alcohol and almost insoluble in all organic solvents excepting pyridine, in which it is very soluble forming a brownish black solution. All methods of getting thevetin in a pure form failed. Ultimately recourse was taken to the study of the genin in order to throw some light on the constitution of thevetin. Thevetidine on simultaneous reduction and acetylation gave a pale yellow micro-crystalline, hygroscopic powder melting at 93°C.

EXPERIMENTAL

The kernels of the nuts contained 22·95 per cent of moisture and weighed 22·6 per cent of the whole nut. The kernels contained 6·4 per cent of a thin light brown coating. The average weight of a kernel was ·35 gm. and it contained 7·4 per cent of moisture.

In order to test the presence of enzymes, the crushed kernels were kept in water. But the presence of oil formed an emulsion which could not be separated. Next time 50 gms of the crushed kernels were put in a flask with petroleum ether for several hours. It was filtered and the oil removed by distilling off the petroleum ether. This was repeated several times ti the kerne s contained no oil The

kernels were then put in an open dish for the petroleum ether to escape The dry powder was then put in a flask with water at the room temperature for three days. Few drops of chloroform were added to stop bacterial growth. It was filtered and ethyl alcohol was added to the filtrate. A white flaky precipitate slowly settled at the bottom showing the presence of enzymes.

10 gms. of the kernels were tested for the presence of alkaloids, but with negative result.

After completely burning the kernels 1 9 per cent of white residue (ash) was obtained, which contained 3 1 per cent of SiO_2. The soluble portion of the ash contained phosphate and magnesium.

For complete analysis 1·5 kilograms of the kernels were crushed and exhaustively extracted with 5 litres of petroleum ether (B.P. 35—60°C.) in a round bottom extraction flask, till the extract no longer gave any oily residue. The total quantity of oil obtained amounted to 1030 gms. which corresponded to 68·6 per cent of the kernels. A current of air was passed through the oil for about 40 minutes to drive off the petroleum ether. For further purification the oil was treated with animal charcoal, little quick lime and Fuller's earth. It was heated over water-bath and stirred for some time. On filtration a very light yellow transparent non-drying oil was obtained. The oil has been worked out by Bhattacharya and Ayyar (loc. cit.).

The refractive index of the oil at different temperatures was determined by means of a Pulfrich refractometer :

Temp.	Observed reading	Refractive index.
10°C ...	42° 44′ ...	1·47195
20°C	43° 15′ ...	1 46889
30°C ...	43° 45′ ..	1 46593
40°C	44° 22′	1 46225
50°C	44 59	1 45856

The kernels were freed from petroleum ether and successively extracted with alcohol the extract gave traces of residue on evaporation The alcoholic extract was concentrated under reduced pressure when a thick brown syrupy liquid, strongly smelling of sugar, was obtained. This slowly solidified to a brown mass in a vacuum desiccator. On extraction with chloroform it gave 45 gms. of yellowish brown solid which was completely soluble in ethyl acetate. Traces of oil that was contaminated with it was removed by petroleum ether. On crystallization from dilute alcohol it was obtained as fine white needles melting at 194°C. It dissolved in strong sulphuric acid with the production of a yellow colour which slowly changed to pink and finally to a cherry-red This product was thevetosin, the water insoluble glucoside. It reduced Fehling's solution readily after being hydrolysed with dilute hydrochloric or sulphuric acids. It was soluble in organic solvents excepting benzene and gave a positive rotation of $[\alpha]_D^{35} = +66{\cdot}85$ in absolute alcohol. On combusting the substance the following results were obtained :

C = 64·95 per cent ; H = 8·22 per cent, and therefore O = 26·83 per cent

Hydrolysis of Thevetosin.—3 gms. of thevetosin was dissolved in 200 c.c. of ethyl alcohol and 150 c.c. of water containing 2·5 c.c of HCl (d. 1·16) was added. It was refluxed for about an hour. The solution was cooled and carefully neutralised with sodium carbonate. It was next concentrated under reduced pressure. A semi-solid brown, sticky substance separated This was the genin—'thevetosidine.' On crystallization from alcohol and animal charcoal it was obtained in the form of a light brown liquid sticky mass which settled at the bottom. After sufficient of the substance had separated the upper liquid was removed and the product was washed several times with distilled water

After few days it became brittle, when it was powdered and put in a vacuum desiccator. It was finally obtained as a yellowish brown micro-crystalline powder melting at 83°C. Concentrated sulphuric acid produced a pink-red coloration with a green fluorescence. In strong nitric acid it dissolved with a yellow coloration. Alcoholic solution of the substance did not give any precipitate or colour reaction with ferric chloride.

The mother liquor after the separation of thevetidine was concentrated and finally evaporated to dryness in reduced pressure. The residue was dried over H_2SO_4 in vacuum desiccator and was extracted with dry acetone. The extract was evaporated to dryness. This was the sugar of hydrolysis It reduced Fehling's solution readily. An attempt was made to crystallize it from ethyl acetate but instead of getting a better stuff, a brown sticky substance was obtained. The quantity being small, the sugar could not be identified.

Thevetin.—The product left after the separation of thevetosin by chloroform extraction contained another glucoside, thevetin, which was very hygroscopic. It contained some free glucose, albumenous product and other water-soluble impurities. All methods of separating the glucoside in a pure form having failed, recourse was taken to the study of the gluco-genin obtained on hydrolysing thevetin.

Hydrolysis of Thevetin.—The same experimental procedure was followed in the hydrolysis of thevetin as in the previous one. Hydrolysis was effected very soon in this case. Thevetidine, the gluco-genin, first separated as a brown semi-solid mass which slowly got oxidised in the presence of air changing colour to green, blue and finally into a black mass. Thevetidine was very little soluble in alcohol and was almost insoluble in all organic solvents with the exception of pyridine in which it was considerably soluble forming a brownish black solution A little of the

substance was dried powdered and washed several times with distilled water and alcohol. It was next dried in the air oven. Its melting point could not be accurately determined. On combustion it gave the following results : C = 90·00 per cent ; H = 9·11 per cent. The sugar isolated from the mother liquor was identified to be glucose.

Thevetidine was next simultaneously reduced and acetylated, when a white product melting at 93°C. was obtained.

Reduced and Acetylated Thevetidine.—5 gms. of thevetidine, 10 gms. of finely powdered zinc dust and 100 c.c. acetic anhydride were put in a dry flask and put under reflux. Few drops of water was added when the evolution of hydrogen started. It was then slowly heated. When the reaction slowed down few drops of water was again added. The operation was repeated several times till the zinc dust was completely used up. It was then filtered hot. The filtrate on dilution and neutralisation with ammonia gave a brown liquid deposit at the bottom which solidified. It was crystallized from alcohol and animal charcoal. A micro-crystalline powder was obtained which melted at 93°C.

The substance gave pink and yellow colour reactions with sulphuric and nitric acids respectively.

On combusting the substance the following results were obtained . C = 60·41 per cent ; H = 6·89 per cent.

INDUCED AND PHOTOCHEMICAL OXIDATIONS AND THEIR IMPORTANCE IN BIOLOGICAL PHENOMENON

BY
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We have been carrying on for the last eight or nine years some work on slow and induced oxidation and have studied their importance in biological phenomenon. In a series of publications[1] from this Laboratory, we have emphasised the wide applicability of induced reactions and numerous reducing agents have been used as inductors. Ferrous and cerous salts and sodium sulphite are inductors which have been largely investigated. Numerous organic compounds and food materials have been oxidised simply by passing air at the ordinary temperature through solutions or suspensions of the substances in contact with freshly precipitated ferrous hydroxide or cerous hydroxide which acts as an inductor Similar results have been obtained with sodium sulphite as inductor. We have induced the oxidation of substances like carbohydrates, fats and nitrogenous substances, etc., by air at the ordinary temperature by mixing the above substances with sodium sulphite, ferrous hydroxide, cerous hydroxide and other reducing agents. The slow oxidation of these reducing agents set up the oxidation of carbohydrates, fats and proteins. We have been able to establish that induced oxidations are of general occurrence and that the mechanism of the oxidation can very well be explained.

[1] Zeit. anorg allgem, Chem., *122*, 146 (1922); *144*, 283 (1925); Jour. Phy. Chem., *28*, 943 (1924), *29*, 376, 799 (1925); *30*, 939 (1926 *32* 1663 (1928) and *34* 711 (1930)

In order to examine the mechanism[1] of these induced oxidations in the presence of ferrous and cerous salts the formation of higher oxides like FeO_2 (Manchot[2]) and Ce_2O_5 (Job[3]) has been assumed; and these higher oxides oxidise the difficultly oxidisable substances like the food materials.

Our experimental results[4] on the oxidation of sodium formate by air in presence of ferrous and cerous hydroxides lend support to the hypothesis of the intermediate formation of the higher oxides.

From our experimental results[5] on the induced oxidation of glucose by air in presence of ferrous and cerous hydroxides, it will be seen that the induction factor, *i.e.*, the ratio of the amount of oxygen taken up by glucose to the amount of oxygen taken up by inductor is as high as 8 or 9. Similar results are also obtained with other reactions. Spoehr also obtained a value as high as 15 for the induction factor. It, therefore, appears that the oxidised form of the inductor, *viz.*, Fe_2O_3 or CeO_2, etc., is also capable of oxidising the acceptor thereby regenerating the original inductor. Hence these induced reactions appear to be partly catalytic in nature but inasmuch as the rate at which the original inductor is regenerated is small as compared with the rate of its oxidation, these reactions belong more to the induced type rather than the catalytic one

These higher values of the induction factors can be satisfactorily explained from the point of view of the generation of ions in the primary exothermal reaction. Thus, for example, a small amount of an inductor is oxidised; some ions will be generated in this exothermal reaction and

[1] Jour. Phy. Chem., *35*, 2043 (1931).
[2] Ann., *314*, 177 (1899); *325*, 93 (1902). *460*, 179 (1927).
[3] Job, Ann Chem. Phys., (7) 20, 207 (1900).
[4] Palit and Dhar, Jour Phy. Chem., *34*, 711 (1930).
[5] Palit and Dhar, Jour. Phy. Chem., *29*. 799 (1925). *30* 959 (1926)

the ions will activate some molecules of the acceptor or the actor or both. These then will react. This reaction being exothermal will in its turn give rise to more ions which will activate some more molecules of the reactants and so on. Thus the oxidation of a small quantity of the inductor brings about the oxidation of a large amount of the acceptor, that is, the slow oxidation of the reducing agents (inductors) set up the oxidation of carbohydrates, fats, proteins and other food materials.

It is well known that the edible substances like carbohydrates, fats and proteins are very readily oxidised in the body, whereas they are oxidised with difficulty by ordinary laboratory reagents. We have carried on our experiments[1] and have been successfully able to induce in the laboratory the oxidation of edible substances like glucose, starch, milk, butter, egg-white, egg-yellow and also the oxidation of other substances like cholesterol, lecithin, glycerol, etc., at the ordinary temperature by passing a slow stream of air in presence of inductors like sodium sulphite, ferrous hydroxide, cerous hydroxide, etc.

It has also been shown[2] that not only fats but carbohydrates and nitrogenous substances are oxidised by hydrogen peroxide and a ferric salt at 37°, volatile aldehydic or ketonic compounds being formed. We[3] have conclusively proved in a systematic manner that fats, carbohydrates and nitrogenous and other organic substances can be completely oxidised into their main end products, carbon dioxide and water, by air with the help of an inductor, ferrous or cerous hydroxide or in presence of sunlight at the ordinary temperatures and we have thus been able to imitate successfully the physiological process of oxidation on which animal

[1] Jour. Phy. Chem., *34*, 711 (1930).
[2] Jour Ind. Chem. Soc. *6*. 617 (1929).
[3] Jour Phy Chem *34* 11 (1930)

life depends It appears therefore that the intermediate iron peroxide, obtained in the case of hydrogen peroxide and ferric or ferrous salts, must be different from that formed with ferrous compounds and oxygen, because the products of oxidation are different in the two cases.

We have also carried on investigations[1] on photochemical oxidation of each of substances investigated in connection with the slow and induced oxidations. It is interesting to note that all these substances—such as glucose, lactose, maltose, starch, glycogen, glycine, urea, glycerol hippuric, and uric acids, formate, tartrate, stearate, oleate, etc., and complex edible substances such as butter, milk, egg-white, egg-yellow, carbohydrates, etc., can be readily oxidised by passing air through them if they are exposed to sunlight without catalyst or inductor. In each of these cases, complete oxidation to carbon dioxide takes place. Even the most complex substances like butter, milk, egg-white, egg-yellow, lecithin, cholesterol, glycerol, etc., are oxidised quantitatively into carbon dioxide and water.

Our results on induced and photochemical oxidations of all the substances investigated conclusively prove the following facts.

Induced Oxidations

(1) The oxidation of fats is retarded by carbohydrates or less powerfully by proteins and to a greater extent by a mixture of carbohydrates and proteins.

(2) The oxidation of proteins is markedly retarded by carbohydrates and fats.

(3) When fats are rapidly oxidised due to the absence of the negative catalyst, glucose, acetone bodies are likely

[1] Palit and Dhar, Jour Phy Chem., *32*, 1263 (1928); *33*, 1897 (1929), *34*. 737 (1930): *34* 993 (1930): and Zeit. anorg allgem. Chem. *191* 150 (1930

to be generated in the body. Apparently the easily oxidisable carbohydrates which act as negative catalyst in the oxidation of fats are necessary for the complete combustion of fatty food materials, the oxidation of both fats and carbohydrates can go on slowly and simultaneously.

The view that diabetes is due to insufficient oxidation of glucose and fats in the animal body, has been emphasised by us in one of our previous publications.[1] In other words, the disease is caused by want of catalysts or enzymes which in normal health exist in the body and help the oxidation of glucose, fats, etc. It seems pretty certain that disappearance of acetone bodies from diabetic urine due to the injections of insulin is an effect caused by the increased oxidation of glucose in the body. We suggested in one of our publications that insulin, extracts of yeast, vitamins, etc., activate the catalysts or the enzymes and consequently act as accelerators in the oxidation of glucose by air in the system. It may be that minute traces of iron or sulphur are present in insulin, extract of yeast, vitamins, etc., and that this iron present in a very reactive state, is the active substance which helps the oxidation of glucose by air. As from our researches, we find that iron is a very important catalytic agent in many oxidation reactions and that iron in some form or other is essential for life and plays an important part in the oxidation reactions in plants and animals. Hence we urge on medical people to treat diabetes by the internal use of iron salts or colloidal iron preparations which will act as an accelerator in the oxidation of glucose and fats by oxygen.

In one of our publications[2] from this laboratory it was suggested that insulin and other allied substances are

[1] Jour. Phy. Chem., *29*, 376 (1925).

[2] Palit and Dhar, Jour. Phy. Chem., *32*, 1663 (1928), and Jour. Phy. Chem. *31* 1259 1927

good reducing agents and are readily oxidised by atmospheric oxygen and the oxidation of these substances induces the oxidation of sugar in the body. We have now been able to substantiate this view by our new sets of oxidation experiments on insulin and glucose. For these experiments a definite volume of air freed for carbon dioxide was passed through an aqueous solution of insulin (B.D.H.) kept at 25° and the amount of carbon dioxide obtained by oxidation of insulin was absorbed by standard barium hydroxide solution and estimated as usual. When glucose is added to the insulin solution and the same volume of air is passed through the mixture glucose is slowly oxidised and this can be shown by estimation of glucose by Fehling's solution, which, however, cannot be reduced by insulin. In this experiment with insulin and glucose, the oxidation of insulin which is readily oxidised by air at ordinary temperature leads to the oxidation of glucose thus corroborating our previous statements.

In several publications,[1] we have emphasised the importance of induced oxidations in understanding the phenomenon of animal metabolism. It has been stated that the readily oxidisable substances like glutathionone and other substances present in muscle and in other parts of the body, are first oxidised by the inhaled oxygen and these oxidations induce the oxidation of food materials. Insulin and other internal secretions also appear to be readily oxidised in the body and these lead to the oxidation of carbohydrates, fats and proteins. It is now well-known that in the treatment of acute diabetes, repeated doses of insulin have to be injected in order to get satisfactory results. Our experiments on the oxidation of insulin by air show that it is used up by the oxidation in the body and thus repeated doses are necessary. Moreover, the

[1] Jour Phy Chem *35* 2043 1931)

oxidation of insulin leads to the oxidation of glucose in the body and this explains the decrease of glucose in the diabetic blood and urine on injection of insulin.

(4) Animal life is assumed to depend essentially on the catalytic activity of the enzymes and iron in the animal body. It is likely that in the animal body, there exist readily oxidisable substances such as enzymes containing traces of iron in complex colloidal condition and the oxidation of these substances induces the oxidation of food materials.

(5) In the animal body, the iron in the blood accelerates catalytically the oxidation of food stuff by the peroxide formed in the body from the inhaled oxygen. When there is a deficiency of iron in the blood, the animal becomes anæmic. At this stage any iron salt preferably of colloidal nature taken in the body, will supply the natural deficiency and the necessary amount of oxidation will take place.

(6) We also suggest that fever is an auto-catalytic reaction. The oxidation of substances like starch, sugar, proteins, fats, etc., by oxygen in the animal body is believed to be catalytically accelerated by the parasites or secretions of bacteria. Hence the amount of heat generated in the animal body for unit time is increased and the phenomenon of fever is observed. Moreover, like all other chemical changes, the amount of oxidation in the animal body for unit time is also increased by the incipient rise of temperature.

(7) From our quantitative experiments[1] on the oxidation of carbohydrates, glycerol, fats and proteins by air in presence of freshly precipitated ferrous and cerous hydroxides and sodium sulphite as inductors, we have shown that the amount of carbon dioxide obtained in these slow oxidations is practically the same as is expected from the

[1] Palit and Dhar. Jour. Phy. Chem., *34*, 711 (1930). Zeit anorg al gem. Chem *191* 150 (1930)

point of view that the carbohydrates glycerol fats and nitrogenous substances are completely oxidised into carbon dioxide and water by passing air at the ordinary temperature. Similarly, Spoehr has obtained considerable amounts of carbon dioxide from the induced oxidation of carbohydrates by air in presence of sodium, ferrous and ferric pyrophosphates. We are of opinion that those results are of importance because these oxidations are of the same type as those taking place in the animal body. Hence we emphasise that in normal health, the food materials taken in the body are completely oxidised into carbon dioxide and water without the formation of intermediate compounds, just as food materials are completely oxidised to carbon dioxide and water when air is passed through their solutions or suspensions in presence of inductors. Intermediate compounds are only formed in the diseased condition of the animal body.

(8) Voit stated " that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that proteins which burns with difficulty outside metabolises with the greatest ease, then carbohydrates, while fat which readily burns outside is the most difficultly combustible in the body." This conclusion was arrived at by Voit from actual feeding experiments on animals. We have obtained quantitative and comparative results[1] on the velocity of oxidation of fats, proteins and carbohydrates by air and thus tried to establish whether fats or carbohydrates are oxidised more readily in the system. Our results show that the order in which they are oxidised in presence of cerous hydroxide are as follows:

Egg-white > egg-yellow > starch > glucose > butter.

In presence of cerous hydroxide, the induced oxidation of fats, nitrogenous substances and carbohydrates follows the same order as stated by Voit.

[1] Jour Phy Chem *34* 711 (1930)

(9) The experimental results[1] show that carbohydrates, proteins, fats and other substances are oxidised in presence of inductors in neutral and alkaline solutions, and the greater the amount of alkali, the greater is the amount of oxidation. Hence we are of opinion that alkaline treatment should prove efficacious in gout, diabetes, beriberi, rickets and other metabolism diseases, because in presence of even sodium bicarbonate, the amount of oxidation of fats, carbohydrates and nitrogenous substances is greatly increased.

Hence all these results on slow and induced oxidation of fats, nitrogenous substances and carbohydrates occurring either singly or in mixtures by air at ordinary temperature are important, because these oxidations are of the same type as those taking place in the animal body.

Photo-Chemical Oxidations and Physiological Experiments

Aqueous solutions or suspensions of the following substances have been oxidised by passing air in presence of sunlight[2] :

Arabinose, cane sugar, galactose, glucose, lactose, laevulose, maltose, starch, glycogen, urea, glycine, α-alanine, hippuric acid, sodium urate, potassium oxalate, sodium formate, sodium tartrate, potassium stearate, potassium oleate, potassium palmitate, lecithin, glycerol, cholesterol, butter, egg-white, egg-yellow and milk Zinc oxide, uranium nitrate and ferric nitrate act each as a photo-sensitiser in the oxidation of the above substances and the amount of oxidation of these substances is greater than that in their absence.

[1] Palit and Dhar, Jour Phy. Chem , *29*, 799 (1925) ; *30*, 93 (1926).

[2] Palit and Dhar, Jour. Phy. Chem., *32*, 1263 (1928) ; *34* 993 (1930) and Ze t. anorg al gem. Chem *191*, 150 (1930

1. Our results[1] also show that the amount of oxidation increases with (i) the intensity of light, (ii) the amount of light falling in the solutions, and (iii) the time of exposure.

2. Dilute solutions of lactic acid, oxalic acid, tartaric acid and citric acid are appreciably oxidised by air in presence of sunlight and the order in which they are oxidised is :

Oxalic > lactic > tartaric > citric.

3. In order to find out whether in presence of sunlight the carbohydrates, fats, and nitrogenous substances are oxidised completely to carbon dioxide or other intermediate products are formed, we have estimated the amount of carbon dioxide obtained in these oxidations in potash bulbs. The amount of oxidation of these substances was also, in all cases, estimated by direct analysis. The experimental results[2] show that the amount of oxidation determined from carbon dioxide obtained is practically the same as the oxidation found out from the direct analysis of the carbohydrates, fats and nitrogenous substances remaining unoxidised. Hence in presence of sunlight, different carbohydrates, fats and nitrogenous substances can be completely oxidised by air at the ordinary temperature into their main end products, carbon dioxide and water. No intermediate compounds are formed in these photo-chemical oxidations. We have thus been able to imitate successfully the physiological processes of oxidations on which animal life depends.

4. Voit in his necrology of Pettenkofer writes : "That the metabolism in the body was not proportional to the combustibility of the substances outside the body, but proteins which burns with difficulty outside metabolises with the greatest ease, then carbohydrates, while fat

[1] Palit and Dhar, Jour. Phy. Chem., *32*, 1263 (1928), *34*, 993 (1930) and Zeit anorg allgem Chem *191* 150 (1930)

[2] Ibid

which readily burns outside is the most difficultly combustible in the body." We have tried to imitate the metabolism taking place in the animal body and have made comparative experiments[1] on the oxidation of butter, egg-white, egg-yellow, starch, glycogen, and glucose by passing air at the ordinary temperature in presence of sunlight. The following results have been obtained :

Egg-yellow	...	60·9	per cent oxidised
Egg-white	..	31·25	,, ,,
Starch	...	38·2	,, ,,
Butter	...	31·8	,, ,,
Glucose	...	13·6	,, ,,

It appears, therefore, that egg-yellow is the most easily oxidisable substance in presence of light, then come starch, egg-white, and butter, while glucose is the least oxidisable. Hence eggs which metabolise readily in the animal body are also easily oxidised by air at the ordinary temperature in presence of sunlight.

5. We have investigated whether the Einstein Law of Photochemical Equivalence is applicable to the photochemical oxidation of carbohydrates, fats and nitrogenous substances in sunlight. The amount of energy absorbed by solutions of carbohydrates, fats and nitrogenous substances was measured with the help of Boys' radiomicrometer. It is interesting to note that the Einstein Law of Photochemical Equivalence is applicable to the photo-chemical oxidation of glucose, lactose and α-alanine by air. The law, however, is not applicable to the photochemical oxidation of glycine by air where about seven molecules react per quantum of light absorbed. These results show that practically colourless one per cent aqueous solutions of glucose, lactose, glycine and alanine can absorb light from the sunshine falling on the solutions.

[1] Jour Phy Chem., *34* 993 (1930)

This absorption of energy leads to the activation of the molecules and their consequent chemical reaction with oxygen in presence of light. When these solutions are mixed with ferric or uranium nitrate, the absorption of radiation is considerably increased and the amount of oxidation is also increased

6. In one of our previous publications,[1] we have shown that appreciable amounts of the compounds of the peroxide type are formed when air is passed through aqueous suspensions of cholesterol, olive oil, butter and many other substances like cocoanut oil, castor oil, linseed oil, mustard oil, etc. It has been also observed that olive oil can be retained in that activated or excited state for a sufficient length of time if kept in the dark but this phenomenon was not observed in a marked degree in the case of cholesterol, as it was found to have lost its active or excited state in the course of a few days. Moreover, appreciable amounts of glucose have been oxidised by mixing the solution of glucose with the exposed cholesterol, olive oil, butter, and other oils respectively, containing the peroxide compounds. Hence it is believed that the anti-rachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxide, which help the oxidation of food materials in the animal body. Substances can acquire anti-rachitic properties when exposed to light only in presence of air and light.

In the light of the observations made we can safely say that when the food materials are exposed to sunlight in presence of air, they take up oxygen forming some peroxide type of compound which can oxidise other food materials when mixed with them. Consequently the addition of the exposed substances to ordinary food stuff facili-

[1] Jour Phy. Chem., *34*, 737 (1930) . *34*, 993 (1930) . Ind. Jour Med Research *17* 430 (1929)

tates the proper ingestion of food materials and produce efficacious results.

7 Sunlight and artificial lights have been used with great success in the treatment of tuberculosis, pernicious anæmia, rickets, etc. In some previous publications[1] we have emphasised the importance of sunlight in the treatment of deficiency diseases and we have observed that rickets, osteomalcia, beri-beri, pellagra, etc., would have been more common in poor tropical countries like India and China, had not the compensating agent—sunlight—been present. This conclusion has been corroborated by our experiments on the metabolism of animals.

8. Having investigated the above facts on the efficacy of exposed oils in oxidising other food materials, we have carried on experiments[2] on the metabolism of pigeons and rats using these exposed and unexposed oils. Incidentally we have also investigated the influence of sunlight and small quantities of colloidal iron preparations, juice of several green leafy vegetables, tomato, etc., in the metabolism of pigeons and rats. For this, different lots of pigeons and rats were fed on polished Rangoon rice which is believed to be entirely devoid of vitamins for about a month. One lot had plenty of sunlight, whilst the other had very little of it. The lot which had sunshine did not show any sign of polyneuritis whilst the other lot not having sunshine developed stomachic troubles first and then acute form of polyneuritis, paralysis and their eyes were highly affected. All the affected pigeons were separated from the rest and kept in sunlight and fed artificially with substances rich in vitamins and containing iron in small

[1] Jour. Phy. Chem., *32*, 1263 (1928); *33*, 1897 (1929) and Chemie der zella und Gewebe., *12*, 217, 225, 317 (1925), *13*, 209 (1926).

[2] Jour Phy. Chem, *32*, 737 (1930), and Ind Jour. Med Research *17* 430 (1929)

loses. They were all finally cured and restored to normal health.

In carrying out feeding experiments upon animals, one cannot but realise that the balance between health and disease, even between life and death, is actually under control The scales are so sensitive that they have been swayed in one direction or the other by the addition or subtraction of seemingly trifling quantities of certain food stuffs. A growing animal provided with plenty of food can be stunted at will, made to decline almost to the point of death and restored with miraculous suddenness by slight readjustment of the diet.

The experiments on the metabolism of animals were further investigated with different lots of pigeons and rats with the addition of following substances mixed with their diet (Rangoon rice) :—

1. Rangoon rice and kept in sunlight, (2) Rangoon rice mixed with juice of green leafy vegetables and kept in diffused light ; (3) Rangoon rice mixed with irradiated oil and kept in diffused light ; (4) Rangoon rice mixed with unexposed oil and kept in diffused light ; (5) Rangoon rice mixed with colloidal iron preparation and kept in diffused light, (6) Rangoon rice mixed with a little quantity of Bajra (Pennisetum typhoideum) and kept in sunlight ; and (7) Rangoon rice mixed with a little quantity of gram and kept in sunlight. The experiment lasted for a month. Our experimental results show[1] that animals receiving normal diet and sunshine, *viz*, Set Nos. 6 and 7, keep very good health. Even if the animals do not get any vitaminous food but only sunlight, they keep good health. Of course, the animals which get irradiated oils were much better than those getting iron preparations and unexposed oils, though they were

[1] Palit and Dhar, Jour Phy. Chem., *34*. 737 (1930), and Ind. Jour Med Research *17* 430 1929)

kept in darkness. Still they were getting inferior in general health to those which obtained sunlight but no vitaminous food. Hence it is found that irradiated oils are not efficacious as the vitaminous food or even as sunlight. It is also found that iron in small doses which as are present in green leafy vegetables is beneficial to health. Our results also show that iron in larger doses is rather harmful to animals and cannot prevent the attack of polyneuritis. All these experiments confirm our view that sunlight acts as a promoter of oxidation of food materials in the body, and normal food with plenty of sunlight is the best for the maintenance of health. We are of opinion that in presence of sunlight the metabolism of the food materials taken in the system is accelerated and that is how the disease is avoided. We believe that by absorption of sunlight, the body cells are activated and greater amounts of oxidation of carbohydrates, fats and proteins take place than in the absence of sunlight. It seems pretty generally accepted that several diseases are caused by defective metabolism. We are of opinion that in these diseases which are caused by the want of proper metabolism, sunlight or artificial light should be highly efficacious, because as we have proved experimentally that the oxidation of fats, carbohydrates and proteins is greatly accelerated by light.

All our experiments on slow and induced oxidations as well as on photochemical oxidations are imitations of Nature's process of oxidation and throw considerable light on the processes of life phenomenon.

Further work in this line is in progress in these laboratories.

SUMMARY

(1) An explanation for the mechanism of induced oxidation has been suggested

(2) Carbohydrates, fats, proteins, food materials, and other organic substances have in presence of inductors been oxidised

(3) The oxidation of fats is retarded by carbohydrates or less powerfully by proteins and to a greater extent by a mixture of proteins and carbohydrates Also the oxidation of proteins is markedly retarded by fats and carbohydrates

(4) The view that diabetes is due to insufficient oxidation of glucose and fats in the body, has been corroborated by our experimental evidence on the oxidation of insulin, which goes to prove that the oxidation of insulin leads to oxidation of glucose in the body This explains the decrease of glucose in, and disappearance of acetone bodies from, the diabetic blood and urine on injection of insulin.

(5) Iron in the blood accelerates catalytically the oxidation of food materials The iron preferably of colloidal nature, when taken into the system, will supply the natural deficiency and the necessary amount of oxidation will take place, thus showing the efficiency of the iron preparations in deficiency and metabolism diseases An explanation that fever is an autocatalytic reaction has also been suggested

(6) Experimental results on the estimation of carbon dioxide prove that carbohydrates, fats, proteins, and other organic substances are oxidised by air at the ordinary temperature in presence of inductor chiefly to carbon dioxide and not to any intermediate products

(7) Comparative experiments on the induced oxidation of fats, carbohydrates, and proteins show that in presence of inductor, the order of oxidation is the same as that obtained by Voit, the eminent physiologist.

(8) An explanation of the internal use of alkali and alkaline carbonates has been suggested based on the increased oxidation of food materials by air in presence of alkali The alkaline treatment

should prove efficacious in gout, diabetes, beri-beri, rickets and other metabolism diseases.

(9) Aqueous solutions or suspensions of the following substances have been oxidised by passing air in presence of sunlight — arabinose, cane-sugar, galactose, glucose, lactose, laevulose, maltose, starch, glycogen, urea, glycerine, α-alanine, hippuric acid, sodium urate, potassium stearate, potassium oleate, potassium palmitate, potassium oxalate, sodium formate, sodium tartrate, lecithin, cholesterol, butter, milk, egg-white, egg-yellow and dilute solutions of citric, tartaric and lactic acids Zinc oxide, uranium nitrate and ferric nitrate act as a powerful photosensitiser in the above oxidations and in their presence the amount of oxidation in each case is greater than in their absence. Our experimental results show that the amount of oxidation increases with (*a*) the intensity of light, (*b*) the amount of light falling on the solutions, and (*c*) the time of exposure.

(10) Experimental results on the estimation of carbon dioxide prove that carbohydrates, fats, proteins, food materials, cholesterol, lecithin, etc , are oxidised by air in presence of sunlight chiefly to carbon dioxide and not to any intermediate product.

(11) Comparative experiments show that order in which the food materials are oxidised in presence of sunlight is as follows :—

egg-yellow > starch > egg-white > butter > glucose.

(12) The Einstein Law of Photochemical Equivalence is approximately applicable to the photochemical oxidations of glucose, lactose and alanine by air.

(13) Experimental results show that appreciable amounts of the compounds of the peroxide type are formed when air is passed through aqueous suspensions of cholesterol, butter, olive, cocoanut, mustard, castor, and linseed oils and some carbohydrates. These peroxides have been estimated by the amount of iodine liberated by them from an acid solution of potassium iodide Moreover, appreciable amounts of glucose have been oxidised by mixing the solution of glucose with the exposed substances containing the peroxide compound. Hence it is believed that the anti-rachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxides which help the oxidation of food materials in the animal body

(14) From the experiments on metabolism of animals, we have proved that sunlight is the best preventive for diseases like

polyneuritis beri beri rickets etc Olive oil exposed to sunlight and air, comes on close second, whereas iron and unexposed oils are harmful to animals The natural food with plenty of sunlight seems to be the best kind of diet for the maintenance of health. In tropical countries, many deficiency diseases are avoided due to sunlight. Hence sunlight and other kinds of artificial lights prove efficacious in the treatment of diseases specially of metabolic origin.

(15) These results (induced and photochemical oxidations) are very important, because these oxidations are of the same type as those taking place in the animal body. The experiments in this investigation are in reality imitations of Nature's process of oxidation of food materials in the animal body.

SECTION III

BOTANY

THE COMPARATIVE VALUES OF VARIOUS FRESH FRUIT JUICE MEDIA IN RELATION TO THE GROWTH OF CERTAIN DEUTEROMYCETES

BY

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INTRODUCTION

The study of the Deuteromycetes under artificial culture has led to the recognition of the fact that most of the organisms are variable. In these variations may take place when subjected to a number of different environmental conditions, but a change of the substratum often markedly affects their growth. Investigations on the effect of different media on the growth of such fungi have resulted in the discovery of several useful media many of which are used for the production or intensification of some particular character of the organism. Thus a starchy medium such as Rice Agar is best suited for the study of colour production by a fungus and Richard's agar has proved to be very favourable for saltations.

Fruits are specially suitable for the preparation of media because of the valuable nutritive substances they contain and the ease with which their juice can be extracted. That is why a large number of fruit juice media are employed in the Western countries for the cultivation and study of the fungi. In this country it had been the practice to prepare media from those fruits only as are used in

the foreign countries such as Prune juice agar apple agar etc., because their effects are known. But though a large number of fruits grow every year in India there has almost been no attempt to ascertain the relative effects of the media prepared from their juices on the growth of fungi. Moreover, as has been pointed out before, useful results are always to be obtained from the study of the suitability of so many widely varying media. According to Brown (5) the problem why one organism grows well in one medium and not on another is of "greatest interest in pathology, as its solution would form a vantage ground for the study of immunity in so far as the latter is based on nutritional factors."

The present paper is an attempt to investigate the relative values of the juices of certain Indian fruits, as media, for the cultural study of four Deuteromycetes.

Materials and Method.—At the beginning the four fungi were freed from bacteria by the method of Brown (4) and single spore cultures were prepared by the triple dilution and poured plate method. These single spore cultures were kept in plugged test tubes and were the source of all subsequent inoculations. For observations petridish cultures were made in triplicate and no character (saltants excepted) has been presented here that did not appear in all of the three plates.

Preparation of the Media.—1. Brown's synthetic medium with starch.

Asparagin	2 gms.
Magnesium sulphate	·75 gm.
Glucose	2 gms.
Potassium phosphate	1·25 ,,
Starch	10 ,,
Agar	18 ,,
Distilled water to make up	1000 c.c

Asparagin and Magnesium sulphate were dissolved separately in boiling water, the volume measured and added to the rest when cool.

2. Red Mulberry Agar (*Morus indica*).—Only the very ripe deep purplish fruits were selected. They were thoroughly washed and the juice was prepared by squeezing through muslin. The seeds, which were rather hard and so did not get crushed, were discarded. The juice tasted sweet but acidic.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

3. Green Mulberry Agar (*Morus alba*).—Only the very ripe fruits were selected and the juice prepared as in the former case. Taste—very sweet.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

4. Water-melon Agar (*Citrullus vulgaris*).—The skin and the seeds were discarded and the juice was pressed out of the pinkish tissue through muslin.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

5. Kakri Agar (*Cucumis utilissimus*).—The fruits were cut into small pieces and without removing the skin or the seeds were crushed thoroughly in a mortar. The juice was extracted out of the pulp through muslin. Taste of the juice—flat.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

6. Kharbuja Agar (*Cucumis melo*).—The skin and the seeds were discarded. The fleshy portion was pounded in a mortar and pressed through muslin. Taste—sweetish

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c c.

7. Phalsa Agar (*Grewia asiatica*). Only the ripe deep purplish fruits were chosen, washed, and pressed through muslin leaving out the seeds and the fibrous parts. Taste—acidic.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c c.

8. Bel Agar (*Aegle Marmelos*).—The fleshy portion adhering to the hard coat and the central part with mucilage and seeds were rubbed against a stretched muslin. A semi-liquid extract came out leaving the seeds and the fibrous parts. Taste—flat and mucilaginous.

Undiluted extract—50 gms. Agar—18 gms.
Distilled water to make up—1000 c.c.

9. Mango Agar (*Mangifera indica*).—The "Sinduri" variety from Madras was employed being the only kind obtainable at the time. The skin was peeled off, the fleshy portion was cut to slices and pressed through muslin. Taste—sweetish.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

10. Lichi Agar (*Gnaphalium lichi*).—Only the sweetest ones were selected. The skin and the seeds were discarded and the juice was extracted by pressing the fleshy portion through muslin. Taste—sweet.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

11. Pomegranate Agar (*Punica granatum*).—The juice was pressed out of the seeds through muslin. Taste—sweetish.

Undiluted juice—100 c.c. Agar—18 gms
Distilled water to make up 1000 c.c

The pH-value, colour of the media, etc., are tabulated below—

Media.	Symbol used.*	pH-value	Colour of the juice	Colour of the media
Brown's starch	Bt	7 0		Pale-olive buff
Red Mulberry agar.	Mr	4 2	Corinthian purple	Walnut brown
Green Mulberry agar	Mg.	7 0	Yellowish white.	Deep-olive buff
Water-melon agar	Wm	6·2	Deep flesh pink.	Pale Cartridge buff
Kakri agar.	Ka.	7 2	Light green	Pale-olive buff
Kharbuja agar.	Kh.	5 6	Pinkish buff	Pale-olive buff
Phalsa agar	Ph.	3 6	Bright Spinel Red	Pale-olive buff.
Bel agar.	Be.	7 0	Buff yellow.	Deep Apricot orange.
Mango agar.	Ma	4 8	Primuline yellow.	Ivory yellow.
Lichi agar	Li	4 5	Pale milk white	Pale-olive buff.
Pomegranate agar	Po.	5 2	Pale cream	Dirty cream white.

To prevent, as much as possible, the decomposition of the compounds present in the fruit juices the media were sterilized by the method of fractional sterilization, which as recommended by Harsberger (8) was done for 20 minutes at 100°C on each of the three consecutive days. In the case of Phalsa agar and Red Mulberry agar the juice and the agar had to be sterilized separately and mixed just before filling up the plates.

For the present study only those fruits were employed as could be obtained fresh from the local market at the time. Brown's starch-synthetic medium was chosen as the standard for comparison. The pH-value determinations were made by the colorimetric method. It is very difficult, if not impossible, to get correct values with this method especially if the solutions are coloured So the results put forward are to be considered not as exact figures but only as closely approximate values. Colour identifications were made as far as possible with the help of Ridgway's (14) book.

* For convenience the media have been referred to by these symbols.

Observations on the cultures on the first six media which were inoculated at the same time to ensure identical conditions, were made at a temperature varying between 89° to 91·5° F. The cultures on the rest five media, which though inoculated later were also done simultaneously, were grown at a temperature of 93° to 95° F.

Fungi used.—The four fungi used in this work were growing saprophytically on various organic debris. Cultures were sent to Dr. Wollenweber to whom the author is indebted for the identification of the species.

The general characters of the fungi as found on the various media are described below.

Fungus No. 6.—*Fusarium incarnatum* (Rob) Sacc = *Fusarium semitectum.* Berk et. Rav. variety *Majus.* Wr.

Mycelium pale pink, hyaline to minutely vacuolate, septate, 3·5—5·2μ thick. Spores-hyaline, slightly curved, ends gradually attenuated, apedicillate, 0 to 3 septate. Septation mode 1. Range of size 7·8 to 25·9 by 3·5 to 5·2 μ. (Plate III, 1.)

Fungus No. 7—*Macrosporium* sp.

Mycelium deep mouse grey, slightly vacuolate, septate, 3·5—6·0 μ thick. Spores dark coloured, stalked, 0 to many septate (about six), muriform, older ones rough-walled. All of the cells and even the stalk may germinate. Range of size 10·4—62·1 by 6·9—16·4 μ (Plate III, 2 and 7.)

Fungus No. 9—*Acrothecium* sp.

Mycelium dark mouse grey, slightly vacuolate, septate, 3·5—5·2 μ thick, spores dark coloured, the two end cells less dark than the inner ones. Spores pearshaped, elongated or bent, the amount of curvature varies. In three septate spores one of the inner cells exhibits a prominent bulging. Olive-brown to blackish grey in colour. 0 to 3 septate. Septation mode 3 but on some media 1. Germination takes place by the hyaline end cells. (Plate III, 5 and 6.) Range of size 6·9—25·9 by 5·2—10·4 μ.

Fungus No 10 *Spicaria* sp

Mycelium Pinkish white, hyaline to mostly vacuolate septate. 3·5—5·2 μ thick. Spores hyaline to vacuolate, oval to ellipsoidal, a septate. Range of size 5·2—22·4 by 2·6—6·0 μ. (Plate III, 3.)

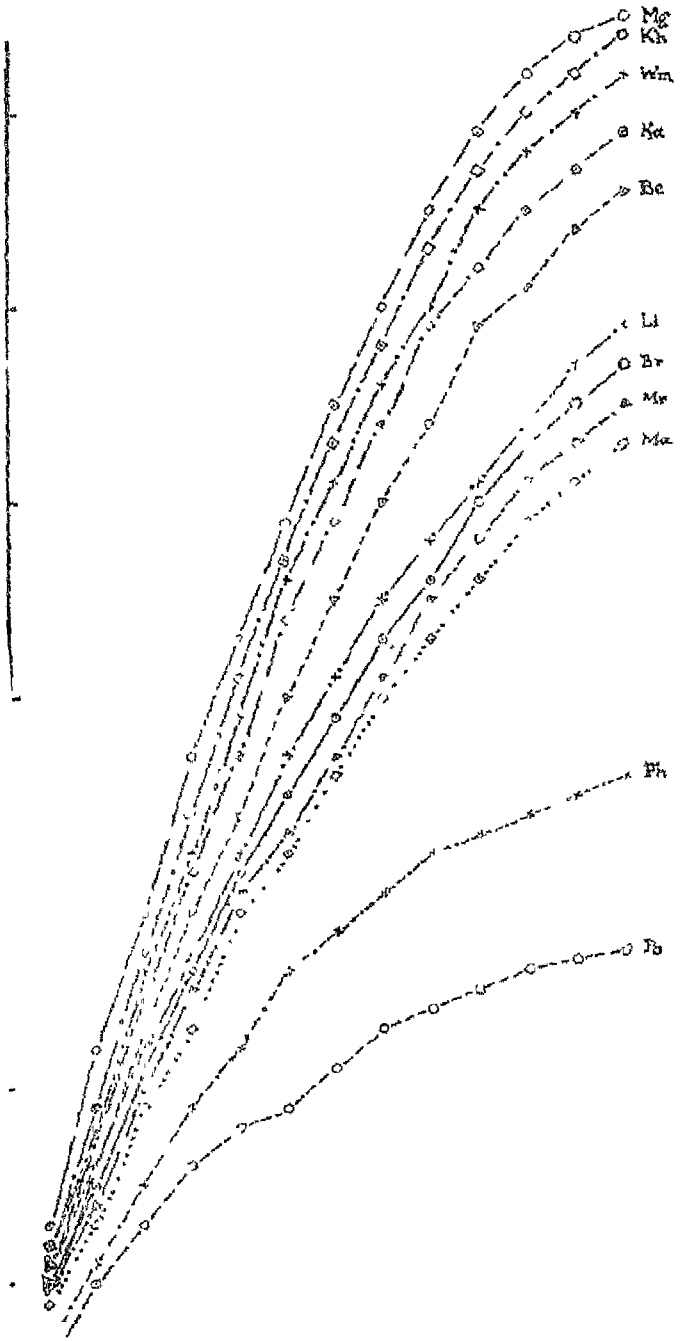
OBSERVATIONS

(A)—Macroscopic Characters

1. *Linear Growth rate.*—From the graph in the Text Fig. 1 it is found that *F. incarnatum* (No. 6) shows the greatest rate of diameter increase on Green Mulberry agar. Its rate of growth on Kharbuja agar is also nearly as great and in fact the average rate of radial advance in both of them is the same (see Text Fig. 5). On Phalsa agar the rate of spread is remarkably slow and on Pomegranate agar it is slowest—the culture not reaching more than 2·7 cms. in diameter even after fifteen days' growth. All the media arranged in a series showing a descending order of growth rate are Mg, Kh, Wm, Ka, Be, Li, Br, Mr, Ma, Ph, Po.

The linear rate of spread of the fungus on all the media gradually falls off as the colony grows and thus gradual staling is shown.

From the graph in Text Fig. No. 2 which shows the growth rate of *Macrosporium* (No. 7) it is seen that for this fungus also the most rapid growth is obtained on Green Mulberry agar and the next rapid growth is found on Red Mulberry agar. The colony on Brown's starch is staling so that though its graph begins at a higher point than many of the media, it later comes down to a lower level. On Pomegranate agar the rate of growth is slowest. The series showing a descending order of growth rate in this fungus is—Mg, Mr, Kh, Wm, Ka, Be, Li, Ma, Br, Ph, Po. The chart for the average rate of radial advance (Text Fig. 5) also shows the same series.


Mg
Ka
Wm
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Li
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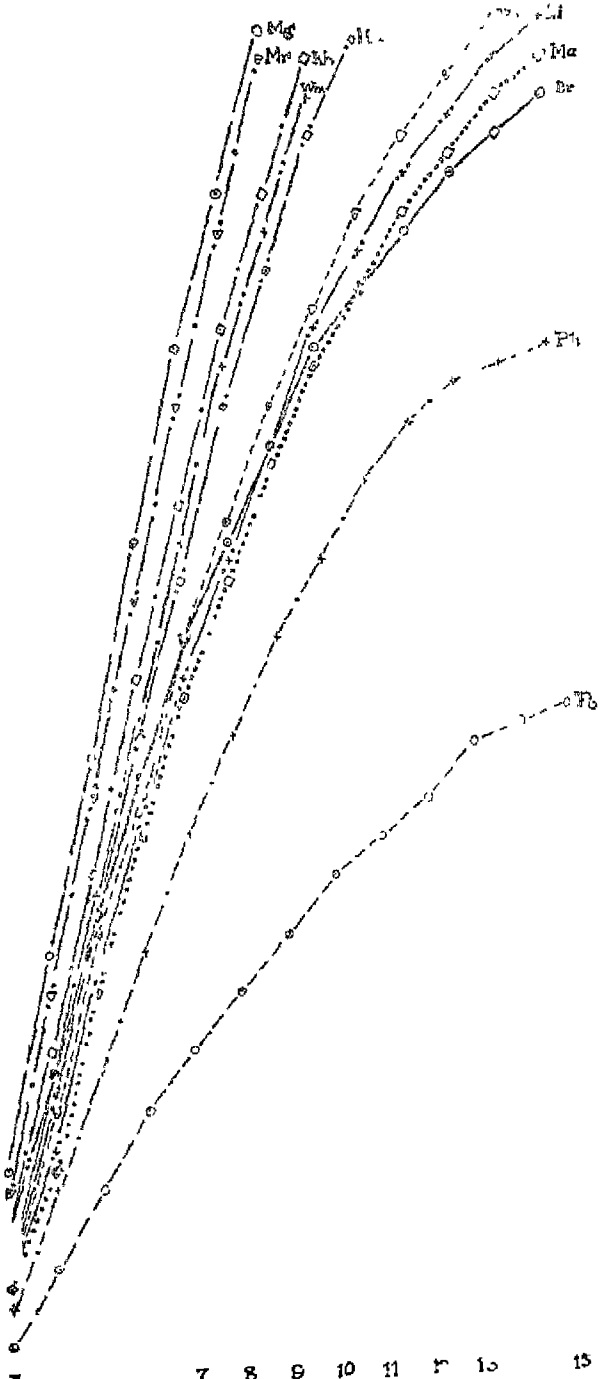
Pb


Mg
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H
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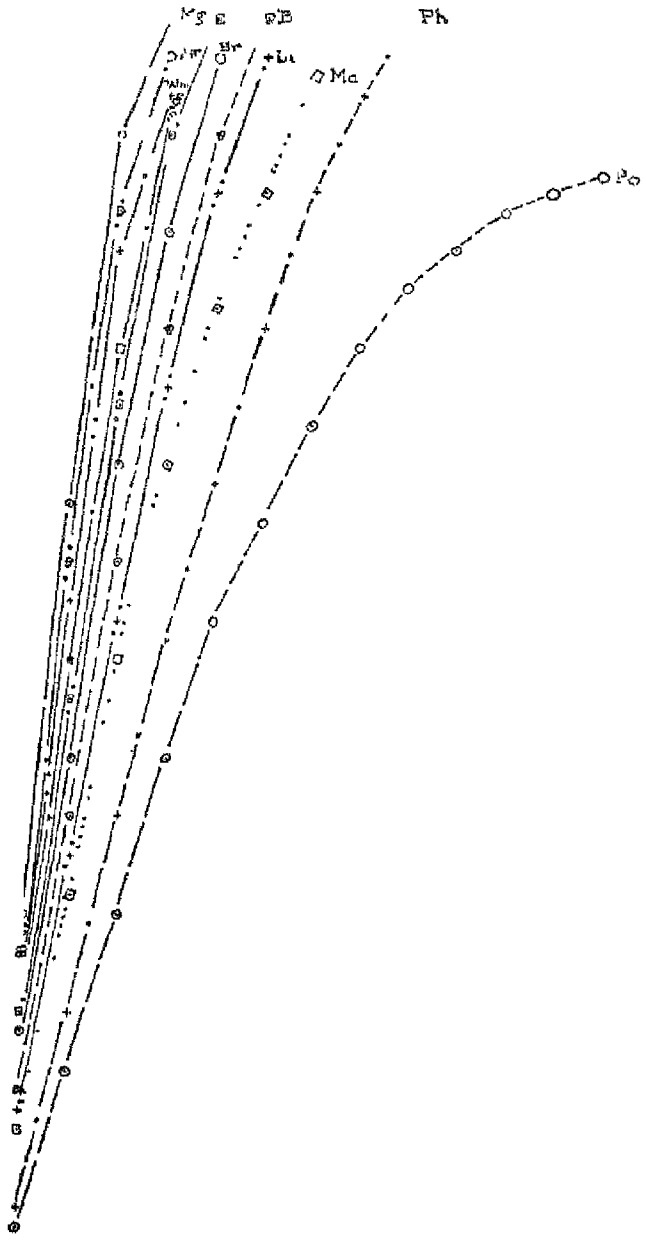
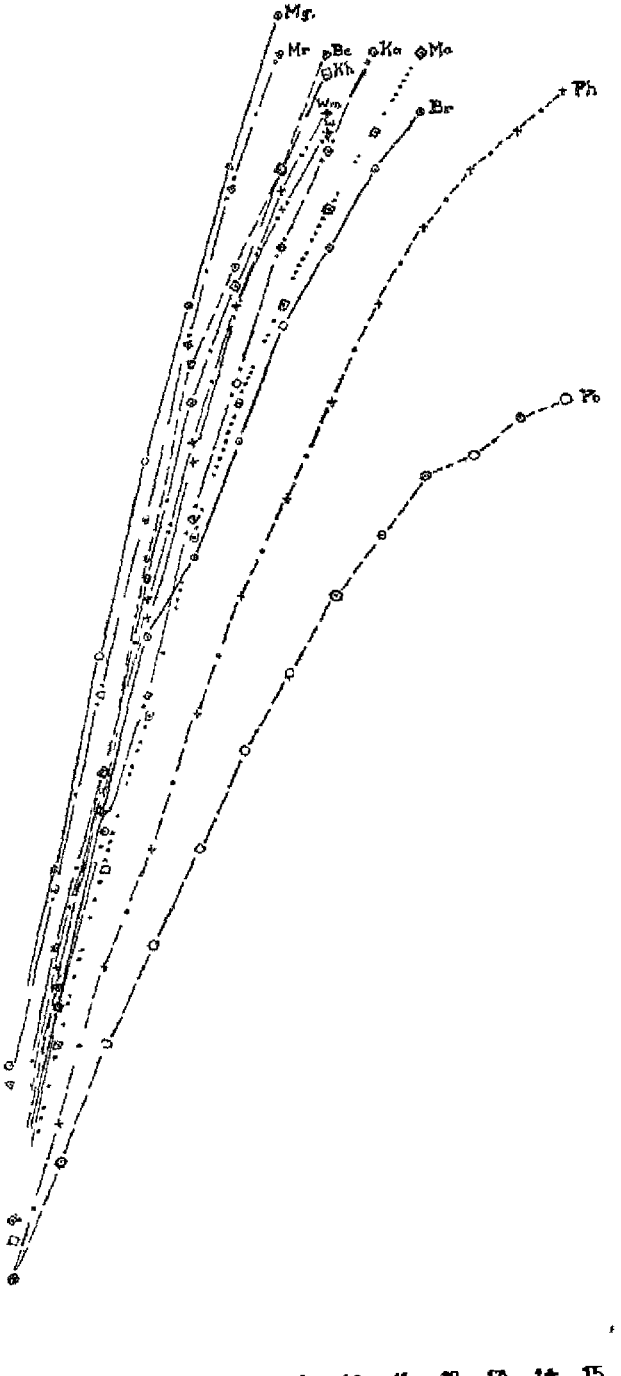
11
12
13
Age of the culture (days)



3 4 5 6 7 8 9 10 11 12 13 14

Age of the culture (days)


Mg.
Mr
Be
Ka
Ma
Kh
Wm
Br
Ph
Pe
0 5 6 7 8 9 10 11 12 13 14 15
Age of the culture (days)


Mg
Wm
Ka
Kh
Ph
Be

The graph in the Text Fig. No. 3 shows that here also ›en Mulberry agar proves to be the medium on which *'othecium* (No. 9) shows the greatest rate of spread. On l Mulberry agar there is second highest rate of growth. ; series of the media showing decreasing growth rate his fungus is Mg, Mr. Wm, Kh, Ka, Br, Be, Li, Ma, Ph Po. The same series is also obtained from the figures he average rate of radial advance (see Text Fig. 5).

In the graph in Text Fig. No. 4 we find that *Spicaria* ›. 10) also shows the greatest growth rate on Green lberry agar. Second highest rate of spread is again wn on Red Mulberry agar. The slowest rate of growth ound on Pomegranate agar. The order showing a des- ding series of the rate of linear growth for the fungus Mg. Mr, Be, Kh, Wm, Li, Ka, Ma, Br, Ph, and Po. e same series also holds good for the average rate of lial advance on these media (see Text Fig. 5).

Macroscopic characters other than the rate of linear ›wth are tabulated below :—

Fusarium incarnatum. No. 6.

Growth	Aerial mycelium	Zonation.	Colour	
			From above	From beneath.
Moderate.	Feeble	2, distinct.	Pale pink.	Light Ochraceous buff
Very fair	Very feeble.	Absent.	Pale cinnamon pink	Darkish Walnut brown
Good.	Moderate.	Absent	Pale cinnamon pink	Near Ochraceous buff.
Slight	Absent.	Absent.	Pale Tellul buff.	Pale Cartridge buff
Scanty.	Absent.	Absent	Pale Tellul buff.	Pale Olive buff
Feeble.	Absent.	Absent.	Pale Tellul buff	Pale Olive buff
Very feeble.	Absent.	Absent	Pale Tellul buff	Pale Olive buff
Very fair.	Feeble	Absent.	Pale cinnamon pink	Near dark Apricot orange
Moderate.	Very feeble	Absent	Pale Tellul buff	Pale Olive buff
Fair.	Very feeble	Absent.	Pale cinnamon pink	Pale Cartridge buff
Very feeble.	Absent	Absent.	Pale Tellul buff	Pale Olive buff

The aerial mycelium has a loose cottony texture where ent On Mr, Li and Ma the aerial mycelium is almost nt but the colony is rather thick. Then comes Kh, , Ka, Ph and Po in order of the thickness of the colony. y are pellucid and have no aerial mycelium. On Brown's- ch only (Plate I, 1) there are two zones of better loped aerial mycelium. The media remain uncoloured he colour as seen from beneath has been recorded.

Macrosporium sp. No. 7.

Growth.	Aerial mycelium	Zonation	Colour	
			From above.	From beneath
Fair.	Fair	3, distinct	Deep mouse grey.	Deep neutral grey.
Good.	Very good	1, broad.	Blackish mouse grey	Dusky purplish grey.
Vigorous.	Abundant	1, indistinct	Blackish mouse grey.	Deep Bluish grey.
Moderate.	Fair	4, faint	Dark neutral grey.	Greyish slate.
Moderate.	Feeble.	7, distinct.	Dark neutral grey	Pale greyish slate.
Moderate.	Good	3, distinct	Deep mouse grey.	Deep slate
Feeble.	Feeble.	1, faint	Dark neutral grey.	Pale slate.
Good.	Very good.	1, indistinct.	Dark mouse grey	Dark slate.
Fair.	Good.	Absent.	Deep mouse grey	Greyish slate.
Fair.	Good	Absent	Dark mouse grey.	Deep slate.
Feeble.	Slight	3, distinct	Deep mouse grey.	Slate.

In this fungus the deep mouse grey cottony mycelium overed over by a whitish mycelium which is mostly ent near the centre. On Mr, it forms a broad near the centre (Plate I, 5) and on Mg and Be rms an indistinct ring at the same place. The growing gin of the colony on Mr, Mg, Be, Li and Br had a Dark green colour. Unlike the previous one this fungus rted colour to the substrata which appeared in a marked ee after about fifteen days' growth. In Br. the colour he medium is present in a prominent ring 1·2 cms wide distance of about 2 cms from the centre.

Acrothecium sp. No. 9

Growth	Aerial mycelium	Zonation.	Colour	
			From above	From beneath
Vigorous	Abundant	1, broad.	Dark mouse grey	Slate grey.
Very vigorous	Very abundant.	3, faint	Dark mouse grey	Dark olive grey
Very vigorous	Very abundant	3, faint	Dark mouse grey	Green bluish slate
Vigorous.	Abundant.	Absent	Dark mouse grey	Slate.
Very good	Very good	4, distinct	Dark mouse grey	Pale slate
Vigorous.	Abundant	3, indistinct	Dark mouse grey	Slate.
Good	Good.	Absent	Dark mouse grey.	Slate grey.
Very vigorous.	Very good.	Absent	Dark mouse grey	Blackish purple slate
Vigorous.	Very good	Absent	Dark mouse grey.	Near castor grey.
Vigorous	Very good.	Absent	Dark mouse grey.	Slate
Fair.	Fair	Absent	Dark mouse grey	Light grey.

In *Acrothecium* the aerial mycelium has an wooly ure and dark mouse grey colour on all the media. Br. there is a broad ring of white surface mycelium 1 the centre (Plate I. 12). On Bel agar there is no ation but the culture shows a number of longitudinal oves extending from the centre of the colony to the edge ate II, 3). The colour noted from beneath is the colour of substrate which is developed to a marked degree after ven days growth

Growth	Aerial mycelium.	Zonation	Colour	
			From above.	From beneath.
oderate.	Fair	Absent	Pinkish white.	Pinkish buff
Good	Fair	Absent	Pinkish white	Dark Walnut brown
Good	Good.	Absent	Pinkish white.	Light cinnamon buff
Feeble	Feeble	Absent	Pale pinkish white	Pinkish Cartridge buff
Feeble.	Moderate	Absent	Pale pinkish white	Pale pinkish buff
Moderate.	Feeble	Absent.	Pinkish white.	Pale pinkish buff.
Very feeble.	Feeble	Absent	Pale pinkish white.	Pale pinkish buff
Good	Good	Absent	Pinkish white.	Dark Apricot orange
Moderate	Moderate	Absent	Pinkish white	Pale pinkish yellow
Fair.	Fair	Absent	Pinkish white.	Pinkish buff.
Very feeble.	Feeble	Absent.	Pale pinkish white.	Pale pinkish white

In this fungus the aerial mycelium is loose and cottony has a pinkish white colour on all the media. On the five media it is developed only in the centre of the ny. The media remained uncoloured but the colour as from below has been recorded.

(B)—Microscopic Characters

As advised by Brown and Horne (7) samples for parative purposes were taken at about 1 cm. ance from the centre when t'e cultures were fifteen old After noting t ie condition (vacuo ation, etc.)

ny chum and th spores the slides were kep
ie and further examinations made.
ie shape of the spores remained practically cons
s been described before The mycelium and the sp
ungi on all the media ranged from hyaline to vacuo
this respect no constancy was observed. In no m
ley granular.

her characters are tabulated below—

Sporulation.

Fusarium	Macrosporium	Acrothecium	Spic m
Feeble	Very good.	Very good	Good.
Very good	Intense	Very good	Intense.
Good.	Very good	Intense	Intense
Feeble	Sparse	Fair	Fair.
Feeble	Fair	Feeble	Fair
Fair	Good.	Fair	Intense
Very sparse	Very feeble	Very feeble	Fair.
Good.	Fair	Very fair	Very good
Fair.	Feeble	Fair	Good
Fair.	Very fair.	Good.	Intense
Very feeble	Sparse	Sparse.	Fair.

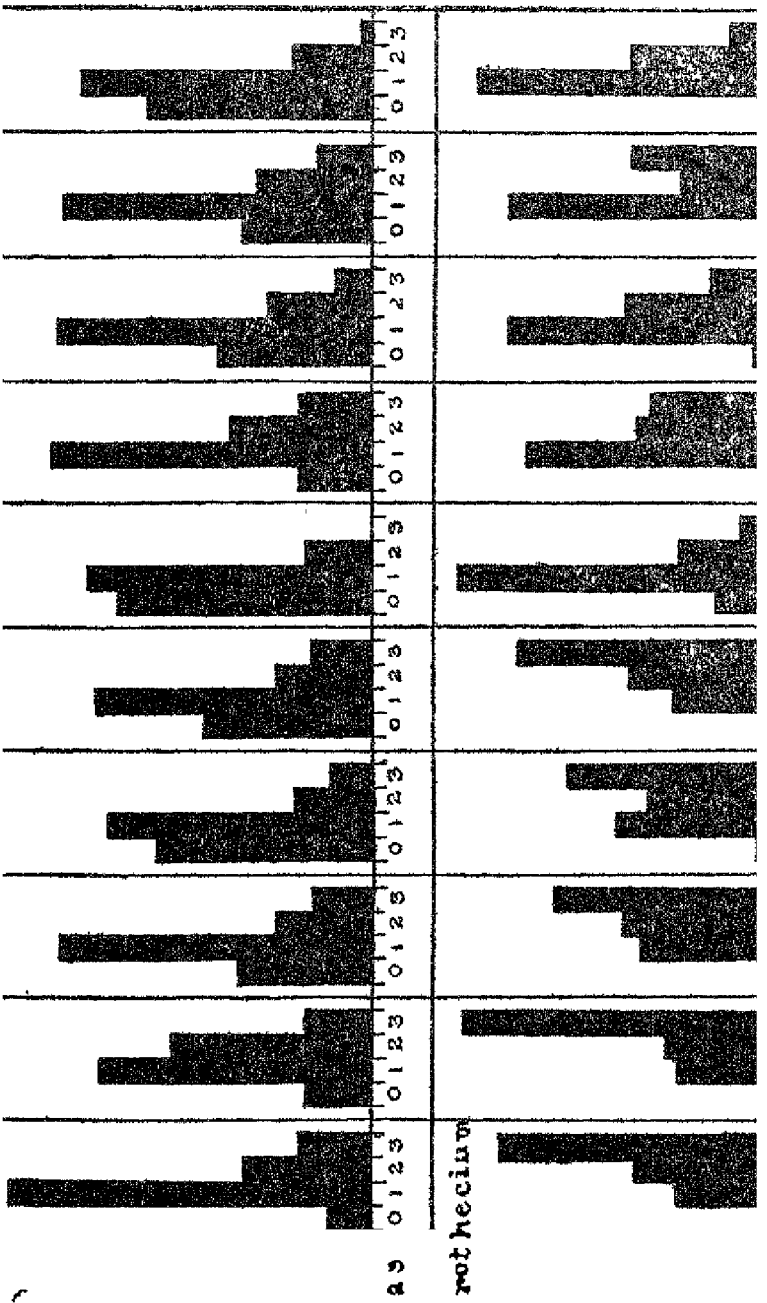
ptation.—The spores were so counted as to a
ious selection. The results of 100 spore counts
graphically in Text Fig. 6, and the average septa
ated below.

Average septation.

	Br	Mr	Mg	Wm	Ka	Kh	Ph	Be	Ma	Li
n	1 0	1 38	1 44	1 14	91	1 08	69	1 35	1 04	1 16
ium	2 14	2 21	2 2.	2 04	2·15	1·94	1·26	1·74	1·51	1·72

ngus No. 7.—*Macrosporium sp.* The spores
m and no counting of septa was attempted

Fungus No. 10.—*Spicaria sp.* The spores were al
septate.



From the text Fig No 6 it is seen that on all the media the septation mode of *F. incarnatum* (No. 6) remains one, but on all of them, excepting Phalsa agar, 0- to 3-septate spores are found. There is greatest number of 1-septate and 3-septate spores on Red Mulberry agar but there is far greater number of 2-septate spores on Green Mulberry agar than on the former medium. So the average septation on the latter medium is higher. If the average septation be the index of the suitability of the different media with regard to this character then the figures give us the following series in a decreasing order of suitability for this fungus: Mg, Mr, Be, Li, Wm, Kh, Ma, Br, Ka, Po and Ph.

On the other hand *Acrothecium sp.* (No. 9) shows a three mode septation on the first six media but on the last five media the septation mode is changed to one. The largest number of 0-septate and 1-septate spores were again found on Phalsa agar which also showed the fewest 3-septate spores. So in the case of this fungus also there is lowest average septation on this medium. On Green Mulberry agar the number of the 3-septate spores and the average septation also are highest. In this fungus the following series of media are obtained from the figures of the average septation. The series in decreasing order is, Mg, Mr, Ka, Br, Wm, Kh, Be, Li, Ma, Po and Ph.

Measurements.—In *Fusarium incarnatum* (No. 6) the largest spores were obtained on Red Mulberry agar and Green Mulberry agar. The 0-septate spores were 9·5—13·8 × 3·5—4·3μ, average size 11·2 × 3·5μ. The 1-septate spores were 14·7—20·7 × 3·5 – 5·2μ, average size 19·8 × 4·3μ. The 2-septate spores were 17·3—22·4 × 4·3—5·2μ, average size 20·7 × 5·2μ. The 3-septate spores measured 19·0—25·9 × 4·3—5·2μ, average size 22·4 × 5·2μ. On Wm, Kh, Be, Ma and Li the next largest spores were obtained. The average size of the 3-septate spores were 19·8 × 5·2μ. On Br, Po and Ph spores of the owest d mensions were found T ie 0-septate

spores ranged from 7·8 11·2×3·5 4·3μ. The 3 septate spores were 17·3—22·4×3·5—5·2μ, average size—18·2×5·2μ.

Macrosporium (No. 7) also shows the largest spores on Red Mulberry and Green Mulberry agars. The measurements are 10·4—62·1×6·9—16·4μ. On the rest of the media the spores generally varied from 10·4—51·8×6·9—15·5μ.

In *Acrothecium* sp. (No. 9) greatest dimensions of the spores are found on the following media—Mr, Mg, Kh, Be, Ma and Li. The spores generally measured, 0-septate ones 6·9—14·7×6·9—8·6μ, average size—13·8×6·9μ. 1-septate spores 10·4—17·3×6·9—8·6μ, average size—15·5×6·9μ. 2-septate spores 13·8—19·0×6·9—10·4μ, average size—16·4×8·6μ. 3-septate spores 17·3—25·9×8·6—10·4μ, average size—20·7×10·4μ. On the rest of the media, *viz*, Br, Wm, Ka, Po and Ph, the spores were shorter and the 3-septate spores measured 15·5—20·7×7·8—9·5μ, average size—18·2×8·6μ.

Spicaria sp. (No. 10) shows largest spores on Red Mulberry agar, the measurements being 6·9—22·4×3·5—6·0μ, average size—13·8×5·2μ. On Mg, Kh, and Be next higher measurements are found—the average size of the spores being 13·0×5·2μ. On Br, Ma, and Li, the average size of the spores is 11·2×3·5μ. On the rest of the media, *viz*, Wm, Ka, Po and Ph the shortest spores are observed. They measured 5·2—7·3×2·6—4·3μ, average size—10·4×3·5μ.

True chlamydospores were found in *Acrothecium* (Plate III, 4). They were absent on Brown's-starch but on other media they were present in fair numbers. Some swellings of the hyphae with no thick wall were however found in almost all the cultures of *Fusarium incarnatum* (Plate III, 8). It is not certain whether they are mere swollen hyphae or are of the nature of chlamydospores.

Saltations.—1. No. 6a.—This saltant of *F. incarnatum* arose as two sectors on Red Mulberry agar (Plate I 2)

It had better developed white aerial mycelium than the parent and probably had a faster growth rate also, because the sectors outgrew the radius of the parent colony. Medium remained uncoloured. Sporulation was very good and the spores were hyaline to vacuolate. The septation mode was 1 and the average septation was 1·30. The measurements were the same as that of the parent.

2. No. 6b.—This saltant (Plate I, 3) of *F. incarnatum* arose on Green Mulberry agar and occupied nearly half of the diameter of the colony. It was very staling and the colony never reached the edge of the plate. The margin of the colony was wavy. There was much less aerial mycelium than the parent. The medium below it remained uncoloured. Sporulation was good, the septation mode was one and the average septation was 1·30. The measurement of the spores were the same as that of the parent

3. No. 9a.—This saltant of *Acrothecium* (Plate I, 12) arose in the form of sectors on two of the three plates of Brown's starch. It had only a very moderate amount of aerial mycelium. The colour of the aerial mycelium was Vinaceous russet (reddish) and in this respect differed very markedly from that of the parent which was dark mouse grey (blackish). Colour of the medium below was light Russet Vinaceous. The red colour of the saltant seemed to be present on the cell wall only. Sporulation was very meagre. The spore mode was 3 and the average septation was 1·96.

The spores were shorter than the parent, their measurements being 3-septate spores 13·8—18·2 × 6·9—9·5 μ, average size—15·5 × 8·6 μ.

4. No. 10a.—This saltant of *Spicaria* (Plate II, 5) arose on Red Mulberry agar and occupied the major part of the culture so that the parent assumed the form of a sector. It had less of aerial mycelium and the colour below was Russet. Sporulation was intense and the spores measured the same as that of the parent.

COMPARISON OF RESULTS AND DISCUSSION

From the observations presented before it is seen that almost all the characters of the four fungi show the greatest development on Green Mulberry agar. The only exceptions are found in sporulation where *Fusarium* and *Macrosporium* show slightly better sporulation on Red Mulberry agar. The measurement of the spores of *Spicaria* is also slightly greater on the latter medium. Zonation, however, is rather poor on Green Mulberry agar. On Red Mulberry agar there is generally second best development of all the characters, though sometimes, as in the case of the development of aerial mycelium in *Fusarium* and *Spicaria* and in the sporulation of *Acrothecium*, it is superseded by other media. Only the linear growth rate of *Fusarium* is rather slow on this medium.

All the fungi in a like manner show very feeble development on Phalsa agar and Pomegranate agar There is least rate of growth of all the fungi on Pomegranate agar and the colour and development of the aerial mycelium is the feeblest. In these respects Phalsa agar is only slightly better than it. But as regards sporulation, septation and measurements of the spores the order is reversed. Phalsa agar proves to be the worst medium and Pomegranate agar is only slightly better.

The rest of the media vary in their positions among the other media with regard to the different characters. On Bel agar there is very good development of most of the characters and often it equals Red Mulberry agar or even Green Mulberry agar. But the linear growth rate of *Fusarium*, *Macrosporium* and *Acrothecium* on this medium is slow. In almost every case Lichi agar comes next to Bel agar but fungi Nos. 7, 9 and 10 show slightly better sporulation on the former medium than on the latter. Not a very good development is shown by the fungi on Mango agar On this medium

their rate of growth is very slow but other characters show a fair development. With these the standard synthetic medium Brown's starch does not compare very favourably. All the fungi show a slow rate of growth on this medium but some characters do show a good development on it. Thus in sporulation of *Macrosporium and Acrothecium* it approaches Green Mulberry agar and Red Mulberry agar respectively. The aerial mycelium of these two fungi shows a fair development on this medium On Kharbuja agar there is very rapid rate of growth but excepting sporulation other characters fail to show a good development on it. Similarly, on Water-melon agar and Kakri agar the fungi show a fast growth rate but all the other characters are developed poorly. Zonation, however, is better shown on Kakri agar than on any other media.

It would be very difficult, if not impossible, to account for all these behaviours of the fungi on the various media It would be hazardous to assign the results to any particular factor in these multi-conditioned metabolic processes This is more particularly so in the event of an almost entire absence of knowledge about the composition of the fruit juices which have been employed However, the following conclusions seem probable.

It has been found that on Green Mulberry agar there is highest rate of spread and best development of other characters but on Water-melon agar and Kakri agar a high growth rate is found associated with a feeble development. Thus it is seen that a greater rate of linear growth does not always correspond to a greater development with regard to other characters. This has also been noticed by Lacy (11) working with the same four fungi. Similar conclusions have also been reached by other workers as Brown (6) and Stevens and Hall (15). The latter state that "no correlation is noted between the rapidity of linear growth and the nutritive value of the medium In many cases most rapid

mean growth occurred in what was surely the poorest medium. It has already been pointed out that this work was carried out at a rather high temperature. According to Balls (1) there is an optimum temperature for growth beyond which the growth curves decrease and Mitra (12) states that for a given fungus the optimum temperature for growth varies with the medium. He finds that Brown's medium gives in general a lower optimum temperature for growth than other media as Prune juice agar, etc. For these fungi also it is possible that the temperature at which they were grown were rather higher above the optimum more especially for Brown's-starch but in the absence of any definite knowledge it is best not to infer any conclusion. Acidity of the medium has a marked effect on the growth of Fungi. Working on *Fusarium* Horne and Mitter (10) obtained curves of the usual optimum type and found that some strains were more tolerant of acid than others. According to Boyle (3) the pH-limits and optimum for growth of *Fusarium* depend on the medium. The results obtained in this work probably justify these conclusions. Both Red Mulberry agar and Phalsa agar have a high concentration of acid but on the former medium there is much better development of all the fungi than on the latter. On the other hand, better development is obtained on the less acidic Green Mulberry agar than on the more acidic Red Mulberry agar. Zonation has been, from time to time, attributed to various causal agencies. Bisby (2) attributes this character to alternating light and darkness and according to Mitra (12) this effect is more clearly marked in the neighbourhood of the optimum temperature. Hedgcock (9) finds that in *Cephalothecium* daily variation in temperature is not the cause of zonation and according to Brown (6) this character is a function of a particular strain and has some systematic value. In this work it is noted that though exposed to the same conditions

af[illegible] ne ned um and not on others fine [illegible] t zones are produced by a particular strain on a [illegible] medium under certain conditions and that no [illegible] can be drawn. Lastly comes the question of [illegible]. As has been noted by others here also it has been found that such characters as the development and colour of the aerial mycelium, sporulation and size of the [illegible], etc., may show a marked change in these sudden variants. The shape of the spores, however, remained the same though in saltants this character [illegible] has been observed by Mitter (13) to vary. As such characters are used in the determination of the species of the genera it is important to exercise great care in doing so.

From the results obtained in this work the author recommends the media in the following decreasing order of suitability for the cultivation of fungi, Green Mulberry agar, Red Mulberry agar, Bel agar, Lichi agar, Mango agar, Brown's sesamum b. Khurbuja agar, Water-melon agar, Kakri agar, Pomegranate agar and Phalsa agar.

In conclusion the writer acknowledges his indebtedness to Prof. J. H. Mitter for the suggestion of the problem and guidance and also to Mr. R. N. Tandon for his help and interest in the investigation.

SUMMARY

1 The effect of eleven media prepared from the juices of fresh fruits on the growth of four fungi, namely, species of *Fusarium*, *Macrosporium*, *Acrothecium* and *Spicaria* has been studied

2 Best development of almost all the characters of all the four fungi is found on Green Mulberry agar and generally the second best development is found on Red Mulberry agar.

3. On the other hand, most feeble development as regards all the characters is shown on Pomegranate agar and Phalsa agar by all the four fungi The other media occupy various positions in the series with regard to the development of various characters The standard synthetic medium, Brown's-starch is much less favourable to the growth of these fungi when compared to many of the media employed.

4 On certain other media as Kharbuja agar, Water-melon agar and Kakri agar a fast rate of linear spread is found associated with a poor development of other characters It is inferred that the rate of linear growth may not give an indication of the amount of growth or the suitability of the medium. This is in agreement with the conclusions reached by other workers.

5 Zonation is found to be produced by a particular strain on a particular medium under certain conditions and no general conclusions could be based as regards the formation of zones either with the individual fungi or the media

6 The nature of the medium is found to be more effective than its pH-value

7. Saltations occurred in *Fusarium* on Red Mulberry and Green Mulberry agars, in *Acrothecium* on Brown's-starch and in *Spicaria* on Red Mulberry agar. Such characters as the development and colour of the aerial mycelium, sporulation and size of the spores are found to be markedly different from those of the parents.

8 From the results obtained the author recommends the media in the following decreasing order of suitability for the cultivation of Fungi—Green Mulberry agar, Red Mulberry agar, Bel agar Lichi agar Mango agar Brown's-starch Kharbuja agar Water-melon agar Kakri agar Pomegranate agar and Phalsa agar

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EXPLANATION OF PLATES I II and III

Illustrating A. K. Mitra's Paper on Comparative Values of Fruit Juice Media.

PLATE I

Fig. 1. F. incarnatum on Brown's-starch. Culture 11 days old.
Fig. 2. F. incarnatum. Parent and Saltant on Red Mulberry agar. Culture 13 days old.
Fig. 3. F. incarnatum. Parent and Saltant on Green Mulberry agar. Culture 20 days old.
Fig. 4. Macrosporium on Brown's-starch. Culture 11 days old.
Fig. 5. Macrosporium on Red Mulberry agar. Culture 11 days old.
Fig. 6. Macrosporium on Green Mulberry agar. Culture 11 days old.
Fig. 7. Macrosporium on Water-melon agar. Culture 11 days old.
Fig. 8. Macrosporium on Kakri agar. Culture 11 days old.
Fig. 9. Macrosporium on Kharbuja agar. Culture 11 days old.
Fig. 10. Macrosporium on Bel agar. Culture 13 days old.
Fig. 11. Macrosporium on Lichi agar. Culture 10 days old.
Fig. 12. Acrothecium on Brown's-starch, Parent and Saltant. Culture 10 days old.

PLATE II

Fig. 1. Acrothecium on Kakri agar. Culture 5 days old.
Fig. 2. Acrothecium on Kharbuja agar. Culture 10 days old.
Fig. 3. Acrothecium on Bel agar. Culture 10 days old.
Fig. 4. Spicaria on Brown's-starch. Culture 11 days old.
Fig. 5. Spicaria on Red Mulberry agar. Parent and Saltant. Culture 10 days old.
Fig. 6. Spicaria on Green Mulberry agar. Culture 10 days old.
Fig. 7. Spicaria on Kakri agar. Culture 10 days old.
Fig. 8. Spicaria on Bel agar. Culture 20 days old.
Fig. 9. Spicaria on Mango agar. Culture 20 days old.
Fig. 10. Spicaria on Lichi agar. Culture 20 days old.
Fig. 11. Spicaria on Phalsa agar. Culture 20 days old.
Fig. 12. Spicaria on Pomegranate agar. Culture 20 days old.

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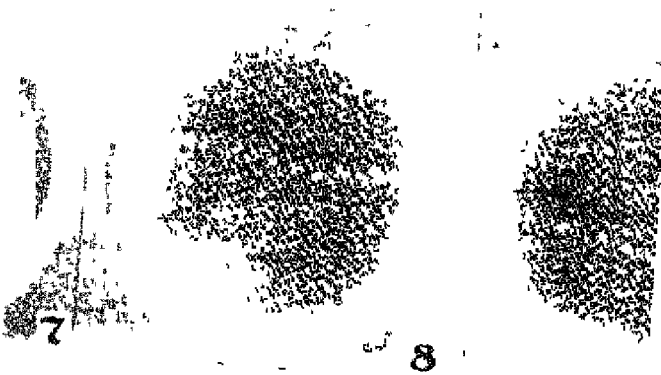

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PLATE III

(All the figures were originally drawn at a magnification of about X1600 with the aid of a Camera Lucida and have been reduced to about X$\frac{1}{2}$)

Fig. 1 Fusarium incarnatum. Spores from Various Media
Fig. 2 Macrosporium Spores from Various Media
Fig. 3. Spicaria. Spores from Various Media.
Fig. 4 Acrothecium. Chlamydospores from Various Media.
Fig. 5 Acrothecium Spores from Various Media.
Fig 6. Acrothecium. Germination of Spores
Fig 7 Macrosporium. Germination of a Spore.
Fig 8 Fusarium incarnatum. Swollen Hyphae on Various Media

SECTION IV

PHYSICS

ON A NEW KIND OF CHARACTERISTIC X-RAYS

BY

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For some time past, the writer of the present note has been thinking of the possibility of having a new kind of characteristic X-rays which stand in the same relation to the usual K and L-spectra as complex optical spectra of elements stand to optical alkali spectra. The K and L-spectra and other usual characteristic X-ray spectra are due to the removal of *one* electron from any closed shell, and the subsequent filling up of this shell by an electron from some external shell according to the rules of quantum mechanics. The alkali-like structure of X-ray spectra is due to the operation of the Pauli principle, according to which *defect* of an electron from a closed shell gives rise to the same spectroscopic terms as excess of one electron outside a closed shell. Is it not just possible that in the act of bombardment by the cathode rays, more than one electron is displaced from one or more shells simultaneously? As such phenomena are quite common in the excitation of optical spectra by cathode ray bombardment, e.g., in Franck and Hertz's experiments, we can expect the same to hold good when we bombard the interior of the atom with electrons of sufficient energy. Supposing now in one act of bombardment, both electrons in the K-shell are carried off, what will happen next? As this state is unstable, two electrons will now jump from the L, or higher shell, and fill up the K-level. It can be shown from principles of quantum mechanics that one of these transitions will be allowed, the other disallowed. But the frequency of the line or lines emitted will be approximately

(K I)+(K I) ionisation appears to double the energy of ordinary K-radiation. Exact calculation shows that there will be two regular lines $^1S_0 - ^1P_1$, $^1S_0 - ^1P_1$ and there may be besides two forbidden lines, $^1S_0 - ^3P_0$, $^1S_0 - ^3P_2$. There may be another group corresponding to K_β, and an intermediate group corresponding to $K_1\ K_1 \longleftarrow L_1\ M_2$ or $K_1\ K_1 \longleftarrow L_2\ M_1$.

It is well known that several lines of obscure origin appear on the shorter side of K lines and are known as spark lines. It appeared to me from scrutiny of existing literature that two at least of these spark lines α_3, α_4 are in reality the double transition lines obtained in the second order. Acting on this hypothesis I directed my colleague Prof Bhargava and my scholar, Mr J. B. Mukerjee to try to get these lines We have to expose our plates at approximately half the wavelength of copper $K\alpha_1$ radiation, and maintain the voltage at a steady value of 40000 which is about double the excitation voltage of $K\alpha$-line of copper. When we developed the plate after twenty hours' exposure a sharp line was found at the expected position impressed on a faint continuous background. Rough measurements showed that it had a wavelength of λ 760 X units while the value of λ for $K\alpha$ of copper is 1530 X units. The measured wavelength is approximately half the expected value, but the measurements were rough, and as the method of fixed crystals was used, there may be large errors in the measurement of the angle. Theoretical considerations show that owing to coupling phenomena the wavelengths of $^1S_0 - ^1P_1$ and $^1S_0 - ^3P$ lines may considerably deviate from the half-value of $K\alpha$ or $K\beta$. The hypothesis of double ionisation and double transition thereby receives good confirmation. About a month previous to this work, working in collaboration with Prof. Bhargava, and Mr. Mukerjee, evidence of double transition L-spectrum of tungsten was obtained

But we postpone announcement of the result till further confirmation was received.

I have no doubt that the phenomenon is general, and before long, workers in this field will be looking for double transition K and L-spectra of all elements. This survey will take much time and labour, but when it is fairly done, there is no doubt that the results will throw a flood of light on the structure of the atom.